



CD4⁺ T cell depletion changes the cytokine environment from a T_H1/T_H2 response to a T_C17-like response in a murine model of atopic dermatitis

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ABSTRACT

Atopic dermatitis (AD) is a common inflammatory skin disease often associated with co-morbidities including allergic hypersensitivity. We have studied induced AD-like disease in NC/Nga mice using the hapten FITC. Following FITC-treatment the NC/Nga mice develop AD-like skin lesions in regard to the histopathological and immunological changes. Consistent with AD in humans the number of CD4⁺ T cells within the blood and draining lymph nodes increases considerably. To evaluate the contribution of T_H cells on disease development we examined the effect of CD4 depletion. Following CD4 depletion the mice still develop AD-like skin lesions characterized by e.g. increased epidermal proliferation, hyperkeratosis and cellular infiltrate, however, the underlying immunological mechanisms change. CD4 depletion results in increased IL-17A and IL-22 production, which traditionally are associated with T_H17 cells. Using confocal microscopy, we demonstrate that epidermal CD8⁺ cells are positive for IL-17A, indicating that these cells are T_C17 cells, the cytotoxic T cell counterpart to T_H17 cells. In conclusion, we show that NC/Nga mice develop AD-like disease following CD4 depletion. This is mirrored by an increased production of IL-17A, which we suggest are produced by T_C17 cells. These findings support that CD8⁺ T cells can play a role in AD.

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1. Introduction

Acute and chronic atopic dermatitis (AD) is characterized by massive infiltration of, in particular, CD4⁺ T cells into the involved skin [1]. The disease is often associated with co-morbidities including cutaneous hyperreactivity to environmental allergens [2]. During the acute phase of the disease T_H2-associated cytokines are expressed, suggesting the importance of the T_H2 response in disease development [3,4]. As the disease progresses into the chronic phase, the cytokine profile changes to a mixed T_H1/T_H2 profile. Although CD4⁺ T cells are considered pivotal for AD development, CD8⁺ T cells are also present in increased numbers. Additionally, in AD patients with house dust mite-associated dermatitis, the level of allergen-specific CD8⁺ T cells correlates to disease severity thus suggesting the importance of this cell type in AD [5].

The NC/Nga mouse is a commonly used model for AD, as it develops an AD-like disease spontaneously when maintained in a

conventional environment [6]. AD-like lesions can be induced under specific pathogen-free (SPF) conditions using haptens e.g. FITC or 2,4,6-trinitrochlorobenzene [7,8].

To determine whether CD4⁺ cells are pivotal for the development of AD-like disease in the FITC-treated NC/Nga mice we examined the effect of CD4 depletion. Interestingly, we observed that disease development was unaltered by CD4 depletion, proposing that CD4⁺ cells are not pivotal for the development of inflamed skin lesions. We then examined the cytokines present in the skin lesions and found increased expression of T_H17-associated cytokines suggesting the importance of these cytokines in the development of disease under CD4-depleted conditions. T_H17 cells, which produce inflammatory cytokines like IL-17A and IL-22 [9], play a role in rheumatoid arthritis, multiple sclerosis and psoriasis [10–12]. Recent findings have revealed a CD8⁺ T_C cell counterpart to T_H17 cells called T_C17, which produces IL-17A and utilizes the transcription factor retinoic acid-related orphan receptor (ROR)γt similar to T_H17 cells [13,14]. T_C17 cells have been associated with the development of autoimmune disease in animal models e.g. experimental autoimmune encephalomyelitis and contact dermatitis [15,16] and recently, the cell type has been found in increased numbers in patients with systemic lupus erythematosus (SLE) [17]. In addition, T_C17 cells are present in

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psoriatic plaques [18], but the role of T_H17 cells in other inflammatory skin diseases remains to be elucidated.

The aim of this study was to describe the effect of CD4 depletion in a murine model of AD. In particular, we wished to evaluate the cytokine production in blood and skin biopsies of e.g. IFN- γ , IL-4 and IL-17A following CD4 depletion.

2. Methods

2.1. Animals

Eight weeks old female NC/NgaTNDcrj mice (Charles River Laboratories Japan, Kanagawa, Japan) were kept in scantainers (5 mice per cage) under SPF-conditions (20–23 °C, 30–60% humidity and light/dark cycle of 12 h/12 h). The mice were allowed to acclimatize for 2 weeks before entering the experiment. Ten mice were included in each group.

2.2. Induction of disease and treatment

AD-like disease was induced in the NC/Nga mice using the hapten FITC, as previously described, with the exception that the mice were challenged once a week for 4 weeks [7]. Mice with fully developed skin lesions were treated daily from day 21 to 32 with betamethasone-17-valerate (Sigma-Aldrich) (7 μ g/ml), a standard treatment for AD [19] or vehicle (acetone). On the days of challenge (days 21 and 28) the steroid or vehicle were applied after a minimum of 1.5 h. All animal procedures were approved by the Danish Animal Experiments Inspectorate (2007/6561-1305).

2.3. Depletion of CD4⁺ cells

To deplete CD4⁺ cells the mice were injected i.p. with 400 μ l of 1 mg/ml anti-CD4⁺ antibody (LEAFTM Purified anti-mouse CD4, Clone Gk1.5, BioLegend, San Diego, CA, USA) 4 days prior to sensitization. An identical concentration and amount of isotype antibody (LEAFTM Purified rat IgG2b κ , Clone RTK4530, BioLegend) was administered to a control group. Additionally, the day before every challenge, the mice were given an i.p. injection of 200 μ l of 1 mg/ml anti-CD4⁺ antibody or isotype control.

2.4. Measurement of skin thickness and transepidermal waterloss

Throughout the experiments, skin thickness was evaluated by skin fold measurements using a micrometer measurer (Mitutoyo, ID-C1012CB, Aurora, IL, USA). Furthermore, transepidermal water loss (TEWL) was determined by a vapometer (VapoMeter, Delfin Technologies Ltd, Kuopio Finland).

2.5. Collection of samples

On day 32 the mice were anesthetized and blood was collected in heparinised tubes by cardiac puncture. The mice were euthanized by cervical dislocation and the draining lymph nodes (ln. axillaris proprius and ln. axillaris accessorius) were removed. The lymph nodes were homogenized using a syringe piston to prepare a single cell suspension for flow cytometry evaluation. Additionally, 2 punch biopsies were collected from the back of the mice. One biopsy was placed in 0.9% saline, homogenized and used for cytokine evaluation. The other was divided into two pieces that were either formalin-fixed and paraffin-embedded or embedded in Tissue-Tek OCT compound (Sakura, Torrance, CA, USA), snap-frozen in liquid nitrogen, and stored at –80 °C until use.

2.6. Histology

The paraffin embedded skin biopsies were cut into 5 μ m sections, deparaffinized and stained with hematoxylin and eosin and analyzed by light microscopy. The histological evaluation was done blinded using a grading scale ranging from 0 (no disease) to 3 (severe disease). The following histopathological changes were evaluated: epidermal hyperplasia, hyperkeratosis, spongiosis, ulceration and cellular infiltration.

2.7. Flow cytometry

Flow cytometry was performed to determine the expression of various cell markers on cells from blood and draining lymph nodes. Red blood cells were lysed by BD Pharm LyseTM lysing solution (BD Bioscience, San Jose, CA, USA), and the remaining cells were washed in FACS buffer (PBS containing 2% fetal calf serum) and blocked for non-specific binding by Fc receptors (Mouse BD Fc BlockTM, BD Bioscience). Antibodies were added to the cells in the following combinations: (1) CD3-PerCP (clone 145-2C11), CD4-FITC (clone GK1.5) and CD25-APC (clone PC61). (2) CD3-FITC (clone 145-2C11), CD69-PerCP-Cy5.5 (clone H1.2F3), CD44-APC (clone IM7), and CD4-PE (clone GK1.5). (3) CD3-PerCP (clone 145-2C11), CD4-FITC (clone GK1.5) and CD8-PE (clone 53-6.7). All antibodies were manufactured by BD Bioscience and used in a dilution of 1/100 (exception: CD4, diluted 1/200). Blank samples were included as controls. Cells were incubated for 20 min on ice, washed twice, fixed in 100 μ l 4% paraformaldehyde in PBS pH 7.4 and examined by flow cytometry (BD LSR II FACS, BD Bioscience). Data acquisition and analyses were performed using BD FACSDiva software (FACSDiva version 6.02, BD Bioscience). For example of gating strategy see Supplementary Fig. 1.

2.8. Evaluation of cytokine production

The presence of the following cytokines in the skin homogenates was evaluated in accordance with the manufacturer's descriptions using Luminex: IL-2, IL-4, IL-5, IL-17A and TNF- α (MilliplexTMMAP, MPXMCYTO-70 K, Millipore, Billerica, MA, USA) or using ELISA: IFN- γ (MIF00, R&D systems, Minneapolis, MN, USA), IL-22 (M2200, R&D systems), IL-23 (88-7230, eBioscience, San Diego, CA, USA) and thymic stromal lymphopoietin TSLP (MTLP00, R&D systems).

2.9. Confocal microscopy detection of CD8⁺ T cells and IL-17A within the epidermis

The co-localization of CD8⁺ T cells and IL-17A within the skin was examined using confocal microscopy. The cryo-preserved tissue was

Table 1

Histological evaluation of the skin in NC/Nga mice. Data are presented as median values. (A) Control mice receiving vehicle show no sign of skin inflammation, whereas FITC-treated mice have increased proliferation of the epidermis and infiltration of cells into the inflamed tissue. Betamethasone reduces the inflammation. (B) Mice were depleted of CD4⁺ cells using an antibody dependent method, whereas control mice received inactivated immunoglobulin of matching isotype. There were no apparent differences between these groups. A total of 10 mice were included in each group.

Histopathological scores H&E staining	Table 1a			Table 1b	
	Control	FITC	Betam.	Isotype control	CD4-depleted
Epidermal hyperplasia	0	2	1	3	2
Hyperkeratosis	0	2	1	2	1
Infiltration of mononuclear cells	0	2	1	2	2
Infiltration of polymorph nuclear cells	0	1	0	1	1
Erosion/ulceration	0	0	0	0	0
Edema/spongiosis	0	1	0	1	0

cut into 10 μm sections and air-dried overnight. The sections were acetone fixed, blocked in 10% standard rabbit serum (X0902, Dako, Glostrup, Denmark), followed by blocking for endogen biotin (X0590, Dako) in accordance with the manufacturer's descriptions. Then the sections were incubated with primary antibodies for 60 min at room temperature (RT). The applied primary antibodies were biotinylated rat anti-mouse CD8 (Clone 53-6.7, R&D System) and Alexa 647-conjugated rat anti-mouse IL-17A (Clone eBioTC11-18H10.1, eBioscience). The biotinylated antibody was detected by Alexa 547-conjugated streptavidin (Invitrogen). Nuclei were visualized using

DAPI. Images of representative fields were acquired using the 488 nm line of a multiline argon laser, the 543 nm line of the green helium–neon laser, and the 633 nm line of the helium–neon laser on a confocal laser scanning microscope (LSM710, Zeiss, Jena, Germany) with a x63 oil-immersion objective.

2.10. Statistical analysis

Statistical analyses were performed using GraphPad Prism 5.0 for MAC (GraphPad Software, San Diego, CA, USA). Kruskal–Wallis test

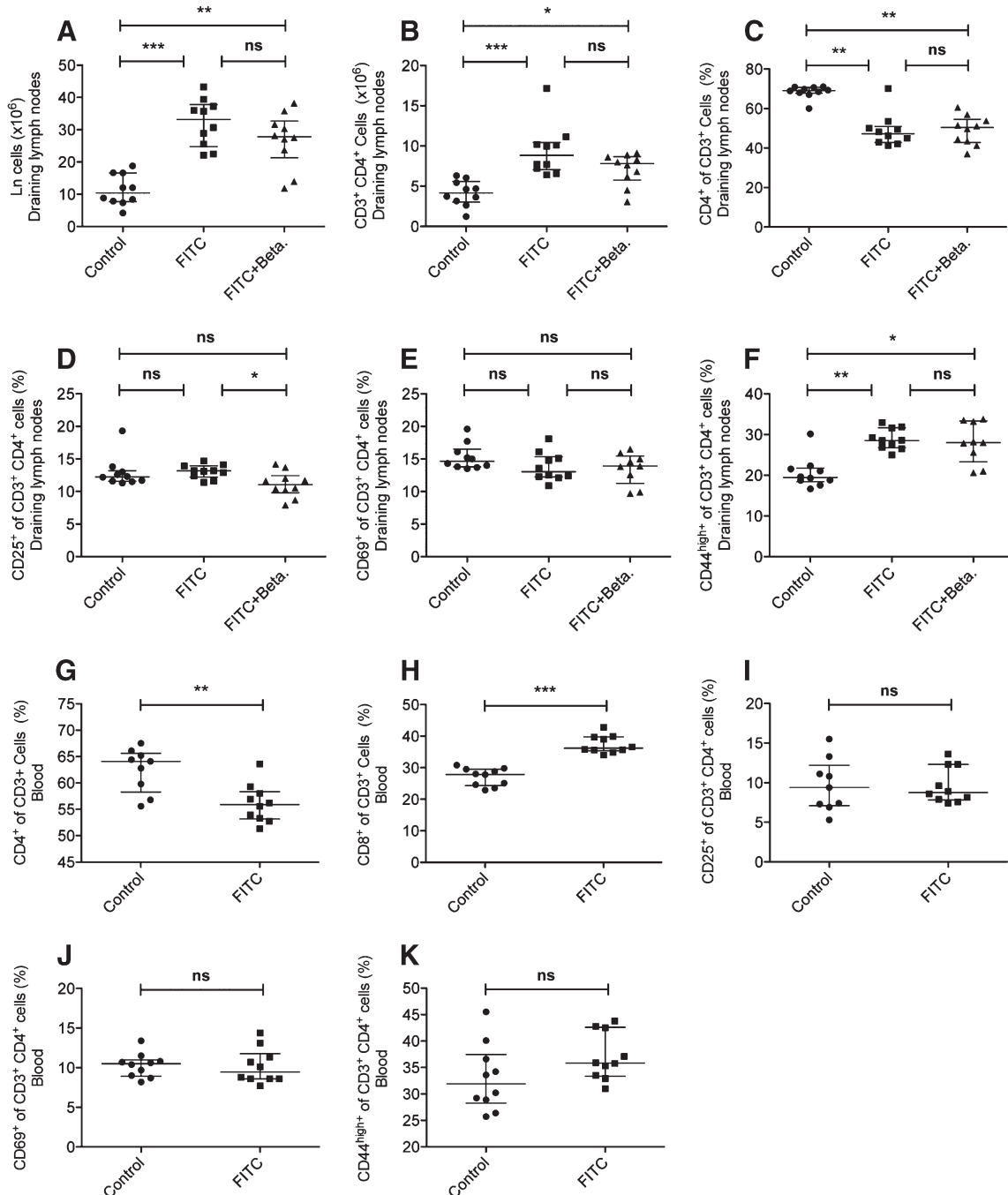


Fig. 1. Evaluation of activation markers on CD4⁺ T cells in blood and draining lymph nodes of FITC-treated mice. (A–C) Although the total number of cells and CD4⁺ T cells increase following disease induction, the proportion of CD4⁺ cells decreases. Results are unaffected by betamethasone treatment. (D–F) The expression of CD25 and CD69 on CD4⁺ T cells in the draining lymph nodes are unchanged following disease induction whereas the proportion of CD44^{high+} effector cells increases. (G–H) Similar to the lymph nodes, the number of CD4⁺ T cells decrease and the proportion of CD8⁺ T cells increases in the blood. (I–K) Additionally, the proportion of CD25 and CD69 expressing CD4⁺ T cells are unchanged in the blood, whereas the percentage of effector cells increases, however, not significantly. Bars represent median with interquartile range (IQR). (ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

with Dunn's multiple comparison post-test was used when comparing 3 groups, and Mann-Whitney *U* test when comparing 2 groups. The value of the detection limit was assigned to samples with an OD measurement below this limit.

3. Results

3.1. FITC-treatment induces an AD-like skin inflammation in NC/Nga mice

FITC-sensitization and challenge resulted in a significant increase in skin thickness ($p < 0.001$) and TEWL ($p < 0.01$) of the NC/Nga mice compared to the healthy control group (Data not shown). Betamethasone treatment reduced skin thickness, whereas transepidermal

waterloss (TEWL) was unaffected or slightly increased (Data not shown).

We used histology to characterize the inflamed dermis further, which revealed a profound inflammation containing both mono- and polymorph-nuclear cells following sensitization and challenge (Table 1a and Supplementary Table 1A). Furthermore, hyperproliferation, hyperkeratosis and some oedema and spongiosis characterized the epidermis, mimicking AD-lesions in humans. Steroid treatment reduced the cellular infiltrate and, in accordance with the measurements of skin thickness, the epidermal proliferation was decreased (Table 1a and Supplementary Table 1A). These results confirm that FITC-treatment induced AD-like skin lesions in NC/Nga mice, in regard to the histological appearance of the skin and responsiveness to betamethasone treatment.

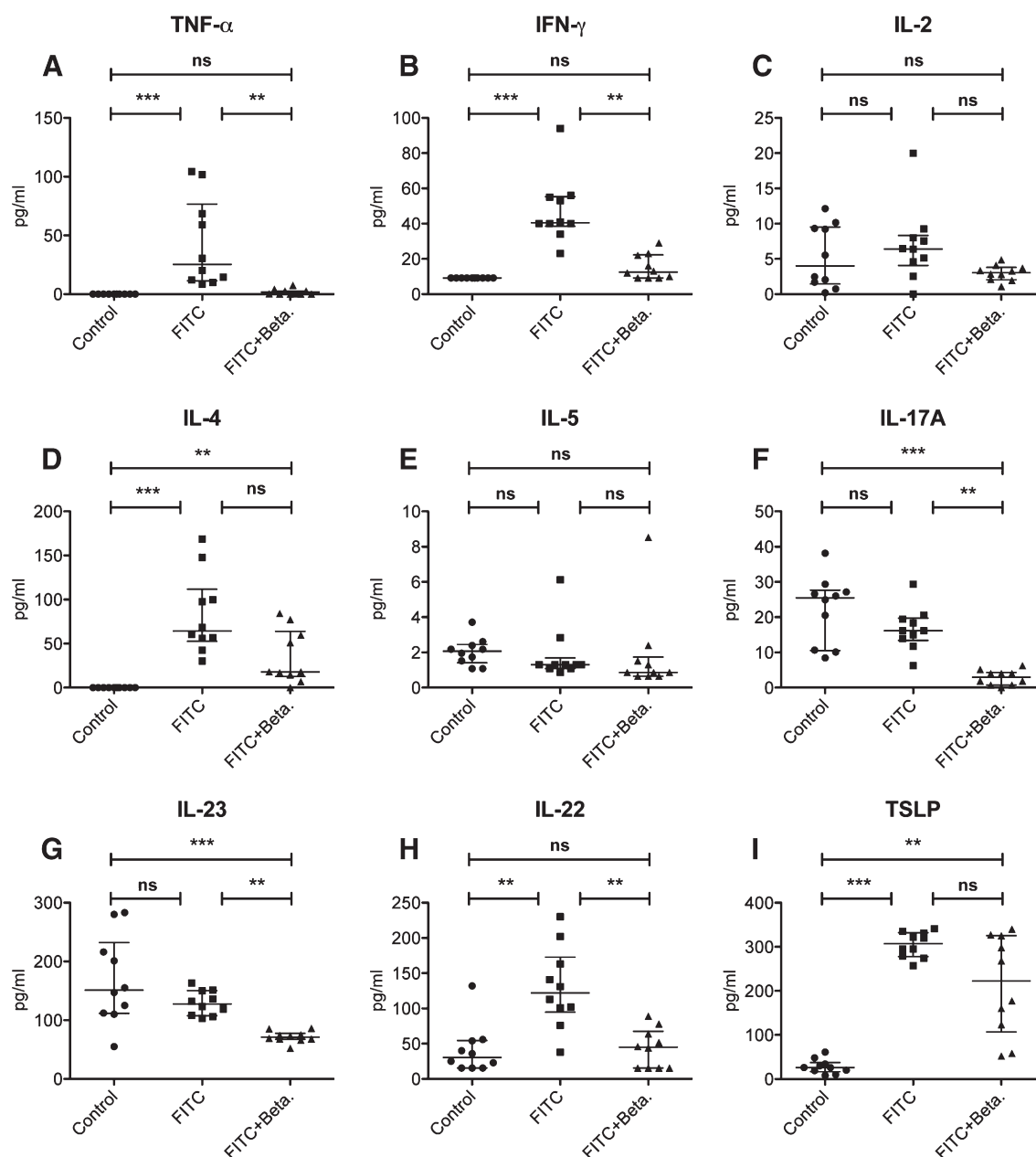


Fig. 2. Cytokine profile of the skin of FITC-treated NC/Nga mice and control mice. (A–C) The T_H1 -associated cytokine IFN- γ and TNF- α increase following disease induction whereas IL-2 remains unchanged. Following betamethasone administration the amounts of IFN- γ and TNF- α are reduced. (D–E) IL-4 increases in lesional skin, whereas IL-5 is unchanged. (F–H) Both the T_H17 -associated cytokine IL-17A and IL-23 are unchanged in lesional skin, whereas IL-22 level increases. (I) TSLP level increases in AD-like skin lesions. Bars represent median with interquartile range (IQR). (ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

3.2. Phenotypic characterization of T_H cells in the draining lymph nodes

After the mice were euthanized on day 32 the draining lymph nodes were excised and the cells further characterized. The total number of cells present in the draining lymph nodes increased following disease induction and remained unchanged after steroid treatment (Fig. 1a). When assessing the number of $CD3^+CD4^+$ cells a similar increase was detected (Fig. 1b). Although the total number of $CD3^+CD4^+$ cells increased following disease induction the ratio of $CD3^+CD4^+$ cells decreased, suggesting that T_H cells migrate into the tissue (Fig. 1c). This observation led us to evaluate the T_H cells ($CD3^+CD4^+$ T cells) for the presence of activation markers. The proportion of cells expressing IL-2 receptor alpha-chain (CD25), a marker for activation, remained unchanged after disease induction (Fig. 1d). This was also the situation for $CD69^+$ T_H cells (early activation marker) (Fig. 1e) whereas the percentage of effector T_H cells ($CD44^{high+}$) increased after FITC treatment, and remained elevated after steroid treatment (Fig. 1f) indicating the presence of a memory T cell population. Corresponding to the results obtained from the draining lymph nodes, the number of $CD4^+$ T cells decreased and the proportion of $CD8^+$ T cells increased in the blood following FITC-treatment (Fig. 1g,h). Furthermore, the ratio of CD25 and CD69-expressing $CD4^+$ T cells in addition to effector cells were unchanged in the blood (Fig. 1i, j, k).

3.3. Evaluation of cytokines present in the skin of the mice

The production of inflammatory cytokines (TNF- α , IFN- γ , IL-2, IL-4, IL-5, IL-17A, IL-22 and IL-23) in the skin of the NC/Nga mice was measured to characterize the local immune response. The FITC-treated mice had a significant increase in the pro-inflammatory cytokine TNF- α ($p<0.001$) and the T_H1 -associated cytokine IFN- γ ($p<0.001$) whereas IL-2 remained unchanged after disease induction (Fig. 2a, b, c). The increase in both TNF- α and IFN- γ was reduced after betamethasone treatment ($p<0.01$). The T_H2 -associated cytokine IL-4

was significantly increased in the FITC-treated mice ($p<0.001$) and the level was reduced, however not significantly, after betamethasone treatment (Fig. 2d). The IL-5 level was unchanged after the induction of disease (Fig. 2e). The T_H17 -associated cytokines IL-17A and IL-23 were present in both the control and the FITC-treated mice in similar levels and decreased significantly in response to steroid treatment in both groups ($p<0.001$, and $p<0.01$, respectively) (Fig. 2f,g). Since the T_H17 -associated cytokine IL-22 has been strongly associated with epidermal changes, we also examined this cytokine in the NC/Nga model. IL-22 level increased significantly after disease induction ($p<0.01$) and decreased in response to betamethasone treatment ($p<0.01$) (Fig. 2h). Finally, we examined TSLP levels, as this chemokine is linked to AD in humans [20]. We found a significant increase in TSLP level following disease induction ($p<0.001$) that was unaltered by steroid treatment (Fig. 2i).

3.4. CD4 depletion and evaluation of skin thickness and TEWL

After having established that T_H cell-derived cytokines increase in response to disease induction we decided to evaluate T_H cells including T_H cell-associated cytokines in the FITC-treated NC/Nga mice. We examined this by depleting the $CD4^+$ cells in the mice prior to FITC sensitization. As a control, a group of mice received inactive immunoglobulin of matching isotype.

CD4 depletion did not affect skin thickness, although a slight increase in TEWL was detected compared to control mice (Fig. 3a,b). Furthermore, the CD4-depleted mice showed a similar degree of inflammation as control mice (Table 1b and Supplementary Table 1B).

Since the CD4-depleted mice developed dermatitis, we evaluated the efficacy of depletion by examining the draining lymph nodes and blood from the mice. The total number of cells in the draining lymph nodes was similar in depleted and non-depleted mice indicating a proliferation of other cell types e.g. $CD8^+$ T cells in the lymph nodes (Fig. 3c). The efficacy of CD4 depletion was evaluated by flow cytometry and only a low percentage of $CD4^+$ cells were present. Of

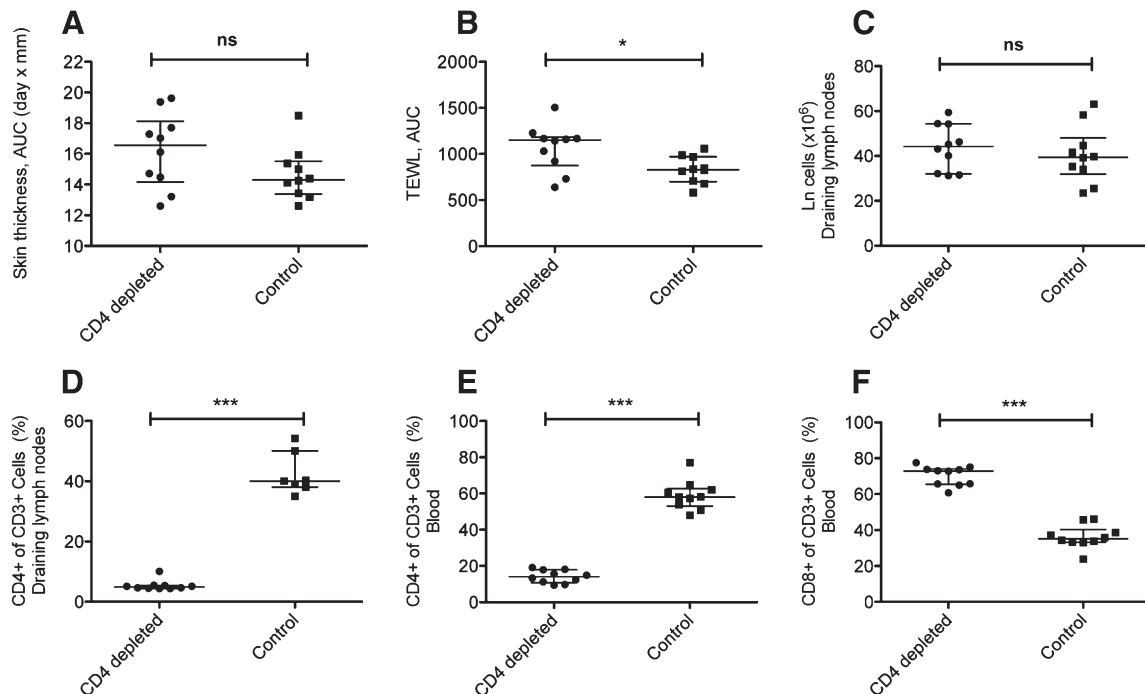


Fig. 3. Evaluation of CD4 depletion. (A–B) Skin thickness and TEWL are unaffected by the CD4 depletion. (C–D) The total number of cells present in the draining lymph nodes is unchanged after CD4 depletion, but the numbers of $CD4^+$ T cells are diminished. (E–F) A corresponding pattern is observed in the blood where only a few $CD4^+$ cells are present following depletion, whereas the ratio of $CD8^+$ cells increases. Bars represent median with interquartile range (IQR). (ns, not significant; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$).

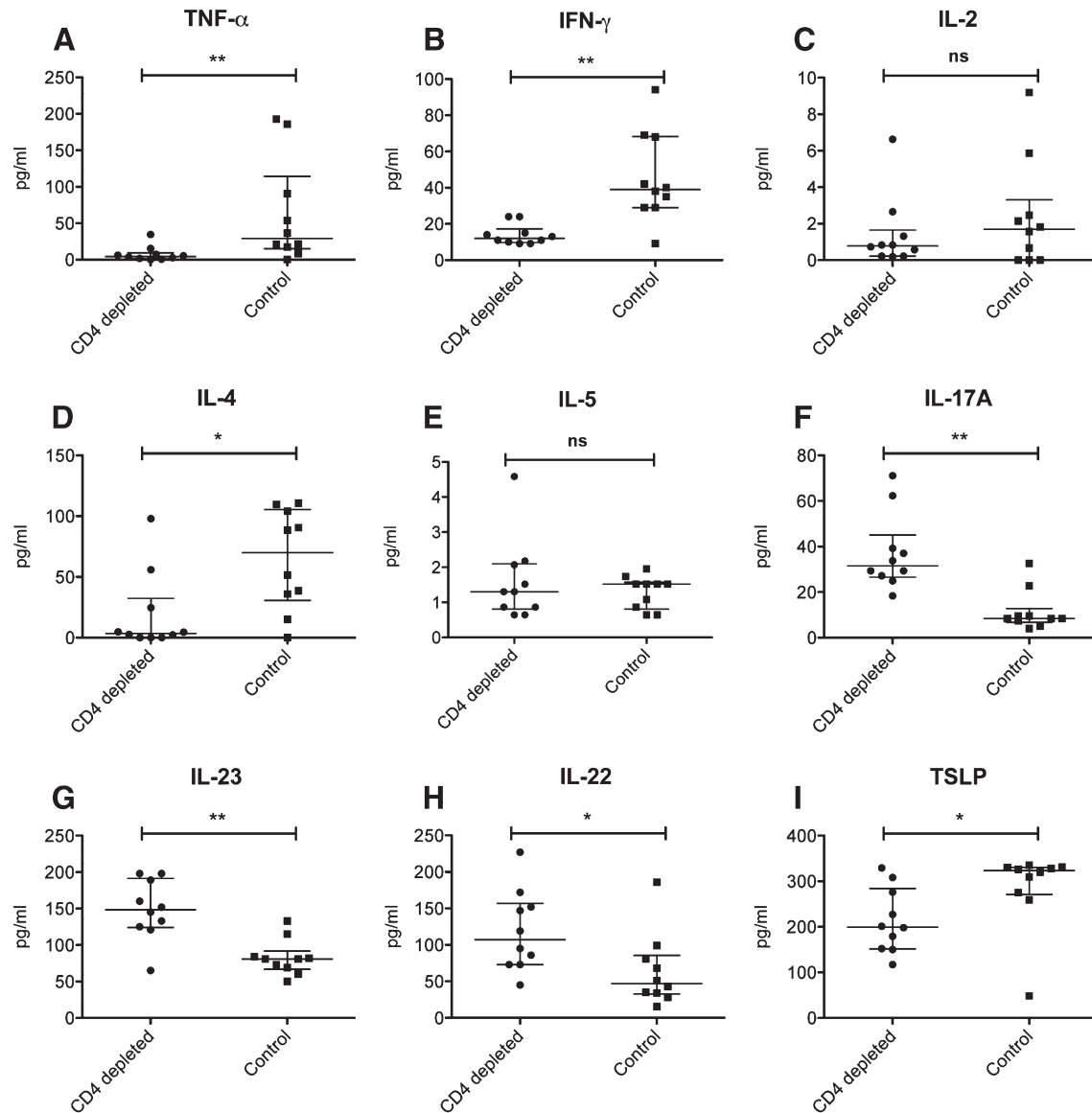


Fig. 4. Evaluation of cytokines present in AD-like lesions of CD4-depleted NC/Nga mice. (A–E) The T_H1 - and T_H2 -associated cytokines (TNF- α , IFN- γ and IL-4) are reduced in skin biopsies following CD4 depletion, whereas IL-2 and IL-5 are unchanged. (F–G) The levels of IL-17A and IL-23 increase significantly following CD4 depletion. (H–I) The level of IL-22 increases whereas TSLP level decreases following CD4 depletion. Bars represent median with interquartile range (IQR). (ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

the $CD3^+$ T cells, less than 5% (4%–5%) were $CD4^+$ in comparison to 40% (38%–50%) in the non-depleted mice (Fig. 3d). Similar results were obtained when examining the blood where approximately 14% (11%–18%) of the T cells were $CD4^+$ compared to 58% (53%–77%) in the non-depleted mice (Fig. 3e). Correspondingly, the proportion of $CD8^+$ cells increased significantly ($p < 0.001$) (Fig. 3f). These results show that even after day 32 only low levels of $CD4^+$ cells were present.

3.5. Evaluation of cytokine production following CD4 depletion

After having established that the CD4-depleted mice developed skin lesions, we investigated the mechanisms behind the development of skin lesions in these mice by examining the cytokines present in homogenates from the inflamed skin.

We found that CD4 depletion resulted in a significant decrease of the proinflammatory cytokines TNF- α and IFN- γ (both $p < 0.01$) in the skin (Fig. 4a,b), whereas IL-2 remained unchanged (Fig. 4c). The T_H2 -associated cytokine IL-4 was also significantly reduced following

depletion ($p < 0.05$) (Fig. 4d). Similar to the previously obtained results the CD4-depleted NC/Nga mice exhibited only limited IL-5 production (Fig. 4e). In contrast to the T_H1 - and T_H2 -generated cytokines the T_H17 -related cytokines IL-17A and IL-23, in addition to IL-22, were significantly higher within the skin of the CD4-depleted mice than in the skin of mice receiving control antibody $p < 0.01$, $p < 0.01$, and $p < 0.05$, respectively) (Fig. 4f,g,h). In contrast to the control mice, the level of TSLP was slightly decreased in the CD4-depleted mice ($p < 0.05$) (Fig. 4i).

3.6. Localization of IL-17A and $CD8^+$ cells within the epidermis of CD4-depleted mice

The continuous production of T_H17 -related cytokines following CD4 depletion caused us to investigate whether $CD8^+$ T cells produced IL-17A in FITC-treated NC/Nga mice. IL-17A-producing $CD8^+$ T cells (T_C17 cells) have recently been associated with the production of IL-17A in psoriasis [12,21]. We therefore performed confocal microscopy to examine IL-17A-expressing $CD8^+$ T cells in the

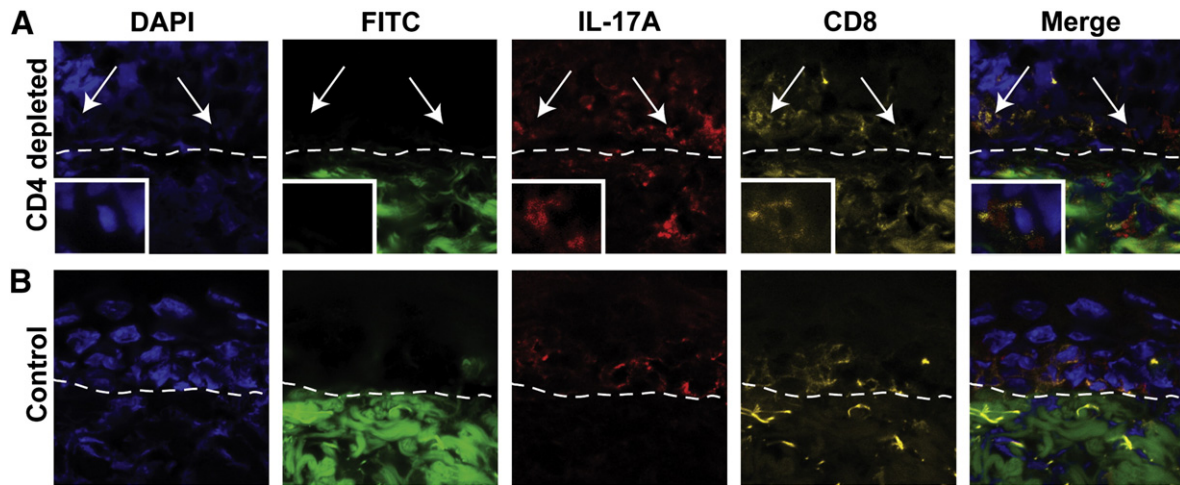


Fig. 5. Co-localization of CD8⁺ cells and IL-17A within the epidermis of FITC induced skin lesions in NC/Nga mice. Representative images acquired using confocal microscopy imaging of epidermis from non-depleted control mice (A) and CD4-depleted mice (B). CD8⁺ cells are visualized as yellow and IL-17A⁺ cells as red. Arrows indicate IL-17A and CD8 double positive cells present within the epidermis of the skin lesions. The hapten FITC (green) is present in the sub-epidermal area and nuclei are visualized using DAPI (blue). The white dotted line indicates the border of the epidermis. The small inserted pictures are close-ups of an IL-17A/CD8 double positive cell in the CD4-depleted mice (right arrow).

skin lesions. This revealed the presence of IL-17A positive cells within the epidermis of both non-depleted and CD4-depleted mice (Fig. 5). Furthermore, double-staining of CD8 and IL-17A revealed co-localization within cells infiltrating the epidermis of the CD4-depleted mice (Fig. 5). Thus, our results show the presence of IL-17A producing T_C cells in the FITC-treated NC/Nga mice. Some cells within the epidermis were only positive for IL-17A. These cells were most likely keratinocytes, since it has previously been shown by others that keratinocytes express the IL-17A receptor on their surface [22].

4. Discussion

We demonstrate that FITC treatment induces an AD-like disease in NC/Nga mice housed under SPF-conditions, as assessed by the physical appearance of the skin and responsiveness to betamethasone treatment. The proportion of CD44^{high}+T_H cells present in the draining lymph nodes increases significantly following disease induction suggesting a proliferation of effector T_H cells, which is in accordance with previous observations in both AD and contact dermatitis in humans.

Furthermore, we observe a reduced ratio of CD4⁺ T cells in blood and draining lymph nodes after disease induction indicating a migration of effector cells into the involved tissue. An increased level of T_H cell-associated cytokines in the involved tissue supports this finding. In particular, we detect increased levels of the T_H1-associated cytokine IFN- γ and IL-4, a cytokine produced by T_H2 cells. The immune response in the acute phase of AD is traditionally characterized as a T_H2-like, whereas a mixed T_H1/T_H2 pattern is observed in the chronic phase (reviewed by [23]). Hence, our results suggest that the immune response in the FITC-treated mice resembles chronic AD.

As expected, the T_H17-associated cytokines are unaltered by disease induction. These cytokines have previously been found increased in psoriasis [8,24,25], but unchanged in AD [26]. However, Nograles et al have recently reported an increased level of IL-22 in both CD4⁺ and CD8⁺ cells within the skin lesions of AD patients, which further supports our data [27].

To characterize further the immune response in this model of induced-AD we CD4-depleted the mice prior to disease induction using an antibody-dependent method. The skin of the CD4-depleted mice resembles the non-depleted mice with regard to the histological

changes, skin thickness and TEWL following FITC-application suggesting that T_H cells are not the sole cause of the pathological changes.

As expected, we found an increased proportion of CD8⁺ T cells following CD4 depletion. CD8⁺ T cells have recently been identified as essential for the development of AD-like lesions in a murine model of allergen-induced AD [28], thus adding to the importance of this cell type in AD. Furthermore, we observed elevated amounts of IL-17A in AD-like skin lesions in mice partially depleted of CD4⁺ cells suggesting the production of this cytokine by a different subset than T_H17 cells.

Recently, a novel T_C17 subset has been described, which is in many respects analog to the T_H17 cells [13,29]. We suggest, that this cell type is responsible for the production of IL-17A within the skin of the FITC-treated CD4-depleted NC/Nga mice. This is confirmed by the presence of IL-17A positive CD8⁺ cells within the epidermis of the skin lesions of the mice.

As CD4 depletion results in an abrogation of the CD4 expressing Treg cells, it may potentially lead to an uncontrolled activation of the inflammatory response of the leukocytes following FITC-application. However, as the mice are CD4-depleted, the uncontrolled immune response will primarily be through the CD8⁺ T cells.

In the light of our results we suggest that T_C17 cells in the FITC-treated NC/Nga mice are, at least in part, responsible for the increase in IL-17A production and we therefore hypothesize a role for T_C17 cell in the development of AD-like inflammatory reaction within the skin of the CD4-depleted NC/Nga mice. The increase in IL-17A occurs following CD4 depletion and after a significant reduction in IFN- γ and IL-4 levels, which supports this hypothesis, since it has recently been shown in mice, that the inhibition of these cytokines significantly increases T_C17 polarization [14]. Thus, we suggest that the CD8⁺ T cells through the T_C17 subpopulation may play a more prominent role in skin inflammation that recognized so far. This is supported by the findings that the depletion of T_H cells, which until now have been considered pivotal for the development of skin inflammation in AD, does not ameliorate the disease, but rather exacerbates the condition. Further supporting the role of T_C17 cells in skin inflammation is that He et al recently reported the presence of T_C17 in a murine model of contact dermatitis [15].

In conclusion, the NC/Nga mouse appears to offer a robust animal model for AD, since the FITC-treated animals exhibit several similarities to the human disease with respect to the pathological findings, response to steroid treatment and some aspects of the immunological response.

In addition, we speculate that T_C17 cells are present within the skin lesions of CD4-depleted mice, further reinforcing the importance of T_C cells in skin inflammation.

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Abbreviations

AD	Atopic dermatitis
FITC	Fluorescein isothiocyanate
IL	Interleukin
ROR γ t	Retinoic acid-related orphan receptor
RT	Room temperature
SPF	Specific pathogen free
TEWL	Transepidermal water loss
TSLP	Thymic stromal lymphopoietin

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Histopathological scores H&E staining	Table Ia			Table Ib	
	Control	FITC	Betam.	Isotype control	CD4-depleted
Epidermal hyperplasia	0 (0-1)	2 (1-3)	1 (0-2)	3 (1-3)	2 (1-3)
Hyperkeratosis	0 (0-1)	2 (1-3)	1 (1-2)	2 (1-3)	1 (1-2)
Infiltration of mononuclear cells	0 (0-1)	2 (1-3)	1 (0-2)	2 (1-3)	2 (1-2)
Infiltration of polymorph nuclear cells	0 (0-1)	1 (1-2)	0 (0-1)	1 (1-2)	1 (0-2)
Erosion/ulceration	0 (0-0)	0 (0-0)	0 (0-1)	0 (0-2)	0 (0-1)
Edema/spongiosis	0 (0-0)	1 (0-2)	0 (0-0)	1 (0-2)	0 (0-1)

Supplementary Table 1: Histological evaluation of the skin in NC/Nga mice. (A) Control mice receiving vehicle show no sign of skin inflammation, whereas FITC-treated mice have increased proliferation of the epidermis and infiltration of cells into the inflamed tissue. Betamethasone reduces the inflammation. (B) Mice were depleted of CD4⁺ cells using an antibody dependent method, whereas control mice received inactivated immunoglobulin of matching isotype. There were no apparent differences between these groups. A total of 10 mice were included in each group. Data are presented as median values and range.