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Measles virus infection of human keratinocytes: Possible link between measles and atopic dermatitis

Geraldine Gourru-Lesimple[a], Cyrille Mathieu[a], Thomas Thevenet[a], Vanessa Guillaume-Vasselin[a], Jean-François Jégou[b], Cindy G. Boer[a], Katarzyna Tomczak[a], Louis-Marie Bloyet[a], Celine Giraud[c], Sophie Grande[d], Catherine Goujon[c,d], Catherine Cornu[c], Branka Horvat[a,*]

[a] CIRI, International Centre for Infectiology Research (Immunobiology of Viral Infections team), Inserm, U1111, University Claude Bernard Lyon 1, CNRS, UMR5308, Ecole Normale Supérieure de Lyon, France
[b] Laboratoire Inflammation Tissus Epithéliaux et Cytokines, Equipe Accueil 4331, Université de Poitiers, Poitiers, France
[c] CHU de Lyon, Service de Pharmacologie Clinique, Service de Pharmacologie Clinique, INSERM CIC1407, Lyon, France
[d] Departement of Clinical Immunology and Allergy, Centre Hospitalier Lyon-Sud, Lyon, France

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ABSTRACT

Background: Measles virus (MV) infection is marked with a skin rash in the acute phase of the disease, which pathogenesis remains poorly understood. Moreover, the association between measles and progression of skin diseases, such as atopic dermatitis (AD), is still elusive.
Objective: We have thus analysed the susceptibility of human keratinocytes to MV infection and explore the potential relationship between MV vaccination and the pathogenesis the AD.
Methods: We performed immunovirological characterisation of MV infection in human keratinocytes and then tested the effect of live attenuated measles vaccine on the progression of AD in adult patients, in a prospective, double-blind study.
Results: We showed that both human primary keratinocytes and the keratinocyte cell line HaCaT express MV receptors and could be infected by MV. The infection significantly modulated the expression of several keratinocyte-produced cytokines, known to be implicated in the pathogenesis of inflammatory allergic diseases, including AD. We then analysed the relationship between exposure to MV by vaccination and the progression of AD in 20 adults during six weeks. We found a significant decrease in CCL26 and thymic stromal lymphopoietin (TSLP) mRNA in biopsies from acute lesions of vaccinated patients, suggesting MV-induced modulation of skin cytokine expression. Clinical analysis revealed a transient improvement of SCORAD index in vaccinated compared to placebo-treated patients, two weeks after vaccination.
Conclusions: Altogether, these results clearly demonstrate that keratinocytes are susceptible to MV infection, which could consequently modulate their cytokine production, resulting with a beneficial effect in the progression of AD. This study provides thus a proof of concept for the vaccination therapy in AD and may open new avenues for the development of novel strategies in the treatment of this allergic disease.

Abbreviations: AD, atopic dermatitis; EGFP, Enhanced Green Fluorescent Protein; H, hemagglutinin; MV, Measles virus; MOI, multiplicity of infection; PFU, plaque - forming units; rec, recombinant; rt, room temperature; TGF, tumour growth factor; TSLP, Thymic stromal lymphopoietin; wt, wild type.

* Corresponding author at: CIRI 1111, 21 Avenue Tony Garnier, 69365, Lyon cedex 07, France.
E-mail address: branka.horvat@inserm.fr (B. Horvat).

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1. Introduction

Measles virus (MV) remains the leading vaccine-preventable cause of child death worldwide [1]. MV is transmitted by respiratory way and causes a systemic infection, with symptoms ranging from respiratory infection, fever and skin rash to less common infections of the nervous system. Patients develop an immunosuppression, which increases their susceptibility to secondary infections, responsible for high incidence of MV-induced mortality [2]. During the incubation period, the virus replicates in the respiratory tract and then reaches the local lymphoid tissues. The amplification of the virus in lymph nodes produces a primary viremia that results in the spread of virus to multiple lymphoid tissues and other organs, including the gastrointestinal tract, liver, kidney and skin [3].

Erythematous and maculopapular skin rash is one of the most characteristic clinical symptoms of measles infection, however, its immunopathogenesis remains unclear and MV infection of skin is poorly understood. Although some studies did not detect MV antigens in affected epidermis [4,5], the others found viral antigens in epidermal keratinocytes and the surface part of the dermis within 6 days of rash [6], as well as in Koplik's spots and noneczematous skin [7,8]. As measles rash appears in the period when the specific adaptive immunity develops, it has been considered to be a mark of the anti-viral immune response, thought to result from skin infiltration by leukocytes, rather than infection of keratinocytes [2].

MV envelope glycoprotein, hemagglutinin (H) was shown to use three different cell surface receptors for entry into host cells: CD46 for vaccine MV strains [9,10] and CD150 and nectin-4, for both, wild type (wt) and vaccine MV strains [11–13]. While CD46 is expressed ubiquitously, CD150 expression is limited to the hematopoetic tissues [14]. The expression of the most recently identified member of MV receptors, nectin-4, has been demonstrated in different tissues, including human epidermis and hair bulbs [15], suggesting thus the possibility for the entry of wt MV in the skin epidermis.

The association between measles and progression of skin diseases, including atopic dermatitis (AD), is still elusive. AD is a highly pruritic skin disease affecting 1–3% of adults, with an increasing incidence in past few decades [16]. MV infection and vaccination have been associated with AD in children by rather contradictory results. While some reports described an increase in the incidence of AD after exposure to measles [17], the others suggested that natural MV infection could reduce the risk of atopic diseases [18] and even highly improve symptoms in AD patients [19,20]. Many immune changes observed during measles also occur after MV vaccination using a live attenuated virus [21,22]. The measles vaccine induces a predominant Th1 response, with IFN-γ as the main cytokine produced in vaccinated children [23]. The MV vaccine also induces a transient immunosuppression in previously vaccinated adults, seropositive for measles, suggesting that the presence of anti-MV immunity does not interfere with the immunosuppressive effect of the vaccine [24].

To better understand the potential association between measles vaccination and infection with the evolution of AD, we performed here more in-depth analysis of the immunopathogenesis of MV infection in the skin. We analysed the implication of skin epidermis in measles and characterized MV infection in human keratinocytes and then tested the effect of live attenuated measles vaccine on the progression of AD in adult patients, in a prospective, double-blind study. We found that MV could infect human keratinocytes and modulates the production of several cytokines known to be important in AD pathogenesis. Moreover, MV vaccination of AD patients was followed by decreased expression of proinflammatory cytokines in their skin biopsies and transient reduction of clinical scores of skin inflammation. These results provide a potential link between the immunomodulatory action of MV infection and the pathogenesis of AD and suggest that measles vaccination not only does not aggravate immunological parameters and clinical signs of AD, but could transiently improve them, opening thus novel avenues for the development of new therapeutic strategies in the AD treatment.

2. Material and methods

2.1. Cells and virus

HaCaT [25], Vero-hSLAM [11] and 293-3-46 [26] cells was grown in DMEM (GIBCO; Invitrogen) supplemented with 10% FCS and antibiotics. Cells were obtained from CelluloNet (Lyon, France), and tested before utilization to be mycoplasma-free using Mycoalert mycoplasma detection kit (Lonza, Switzerland). Primary human epidermal keratinocytes were obtained from surgical samples of healthy breast and abdominal skin as described [27] and cultured to 80% of confluence in Keratinocyte serum-free medium supplemented with bovine pituitary extract (25 μg/ml) and recombinant epidermal growth factor (0,25 ng/ml, Invitrogen Life Technologies, Cergy Pontoise, France).

Recombinant MV IC323 wt and vaccine strains, expressing respectively MV wt or Edmonston H and EGFP [28], were kindly provided by Dr Y. Yanagi (Kyushu University, Japan). MV ROUVAX (Pasteur Merieux Connaught France), containing MV Schwarz strain, was provided by B. Soubeyrand (Aventis Pasteur MSD, France). Recombinant MV Schwarz strain, expressing EGFP was generated using pB(+)Mor-EGFP[6] plasmid, produced by introducing the EGFP gene sequence into a new transcription unit located between the H and L genes in the pB(+)MVvac2 plasmid [29]. Schwarz MV (Mor-EGFP [6]) was rescued in 293-3-46 cells as previously described [26]. Viral strains were produced on Vero/hSlam cells and infections were performed at MOI = 1. In some experiments viruses were inactivated exposure to 254 nm UV-irradiation at 4 °C during 30 min.

2.1.1. Flow cytometry

Cells were stained for membrane expression with anti-CD150-PE, and anti-CD46-FITC (BD Pharmingen) or anti-Nectin-4-PE (R&D Systems) in PBS with 1% BSA for 30 min. Viable cells were acquired on FACSCalibur 3C cytometer (BD Biosciences, Belgium) and FACS analysis was performed using CellQuestPro (BD Biosciences) followed by FlowJo (Tree Star Inc., USA) analysis.

2.1.2. Clinical study

The clinical study was a randomized double-blinded study, performed between January 2009 and March 2012, at the Clinical Research Unit of Immunology at Lyon Sud Hospital (Lyon, France). The study included 20 adult patients (8 women and 12 men), average age 39,9 years old (SD ± 14 years), with moderate AD SCORAD of at least 15. The primary objective was to analyse the effect of measles vaccine on AD physiopathology during 6 weeks of study. Patients did not receive any systemic steroids or other immune suppressive medication 3 months before and during the study. The use of local emollients was allowed. The protocol was approved by the local ethics committee (Comité de Protection des Personnes de Lyon Sud Est II, N° IRB00009118) and written informed consent was obtained from each patient before enrolment. The study was conducted in accordance with the Declaration of Helsinki Principles and its amendment, and was registered in the ClincalTrials.gov number (NCT 00820820) and in the European Clinical Trials Database (EudraCT 2007-007267-25). Study data were computerized by the Investigation Clinical Centre of the Hospices Civils de Lyon.

2.1.3. Anti-measles vaccination and seropositivity test

Patients were randomized using permuted-block algorithm to receive either placebo composed of physiological serum (N = 11) or a single subcutaneous injection in the upper arm region of commercially available ROUVAX (N = 9), composed of the attenuated Schwarz strain, for measles vaccination (≥1000 DICC 50, Laboratory Aventis-Pasteur MSD, Lyon, France). Presence of MV-specific IgG and IgM antibodies in the serum was tested before vaccination and two weeks after, using measles-specific antibodies by commercial ELISA (Enzygnost Anti-Masern-Virus, DADE Behring).

2.1.4. Severity scoring of AD

The clinical severity of AD was determined using SCORAD [30], taking into account the total affected skin area, the intensity of skin lesions and the subjective symptoms including pruritus and sleep loss, with maximum score 103.

2.1.5. Atopy patch and tuberculin sensitivity test

Delayed type hypersensitivity was measured using atopy patch and tuberculin sensitivity tests. Atopy patch test was performed and interpreted according to the International Contact Dermatitis Group guidelines, as described [31]. Briefly, set of patches containing following allergens: timothy, wormwood, cat, flour, date fruits, plantain and *D. pteronyssinus*, (Stallertest–Stallergène), were applied on the dorsal skin of a patient. The response characterized by an erythematous reaction and induration was measured by score ranging from 1 (no reaction) to 7 (extreme reaction). For the tuberculin sensitivity test, Purified Protein Derivate (Tubertest, Sanofi Pasteur MSD, France) was administrated intradermaly, on the volar surface of the forearm, using classical Mantoux technique. The response was characterized 72 h later by measuring the induration diameter.

2.1.6. Skin biopsies

Four-mm skin biopsies were taken under local anaesthesia (Xylocaine 1%) from non involved skin and positive atopy patch test skin, performed on the dorsal skin, on days 0 and 10 after vaccination. Biopsies were placed in the RNALater solution (Qiagen, Venlo, Netherlands) and kept at least 24 h at +4 °C, before being stored at −80 °C, until being further processed for the RNA analysis.

2.2. RNA extraction

RNA was extracted from cells using NucleospinRNA kit (Machery Nagel) and from skin biopsies using QIAzol kit (Qiagen). Skin samples were stored in RNA*later* (Qiagen) at −80 °C until their mechanical disruption with QIAzol Lysis Reagent. After the addition of 200 μl of chloroform, the solution was mixed, incubated for 3 min at rt, centrifuged at 4 °C/12000*g* for 15 min and supernatants were collected and mixed with 70% ethanol. RNA was cleaned using RNeasy Mini Kit (Qiagen), including the additional step with RNase-free DNase (Quiagen) and total RNA were diluted in 30 μl of RNase-free water and stored at −80 °C before use.

2.2.1. RT-qPCR

RNA (0,5 μg) was reverse-transcribed into cDNA using iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Berkeley, California, USA), random hexamer oligonucleotide primers and a T-GRADIENT PCR thermocycler (Biometra GmbH, Goettingen, Germany). The cDNA were diluted 1/10 in DNase-free water and stored at −20 °C.

Quantitative PCR was performed for all cDNA samples with FastStart Universal SYBR Green Master (Roche, Basel, Switzerland) and run on the StepOne Plus Real-Time PCR system (Applied Biosystems, Foster City, CA, USA), using following cycles: 95 °C 10 min, followed with 40 cycles of 95 °C for 15 s, 60 °C for 30 s and 72 °C for 20 s. All samples were run in duplicate and results were obtained with the StepOne™ Software v2.1 (Applied Biosystems,). Quantitative PCR was performed using the following primers: CCL26 forward: GCCGTCTCAGTCTCATAAAAGGG; CCL26 reverse: GTATTGGAAGCAGCAGGTCTTGG; TSLP forward: GTATTGGAAGCAGCAGGTCTTGG; TSLP reverse: GTCTTACCTACTTTTCTATCCCCATTG; TGF-β1 forward: TGGACACCAACTATTGCTTCA; TGF-β1 reverse: GGCAGAAGTTGGCATGGTAG; CCL11 forward: ACCAGAGCCTGAGTGTTGC, reverse: ATGCCCTTTGGACTGATAATGAG; CCL17 forward: CGGGACTACCTGGGACCTC, reverse: CAGTTCAGACAAGGGGATGGG; CCL27 forward: CTCTACCGAAAGCCACTCTCAG, reverse: GCCAGGTGAAGCACGAAAGC; The results were normalized using the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using primers: hGAPGH forward: CACCCACTCCTCCACCTTTGAC; hGAPGH reverse: GTCCACCACCCTGTTGCTGTAG. Calculations were done using the $2^{\Delta\Delta CT}$ model, and according to the MIQE guideline, as described [32].

2.2.2. Statistical analysis

Statistical analyses were performed using the GraphPad Prism software (GraphPad Software, Inc., La Jolla, USA). Data were analysed using the Mann-Withney U-rank test or repeated measures ANOVA, there is no alpha risk adjustment despite the multiplicity of tests. Differences were considered to be statistically significant when $P < 0,05$.

3. Results

3.1. Human keratinocytes express MV receptors and are susceptible to MV infection

Primary human keratinocytes and the spontaneously immortalized human keratinocyte cell line HaCaT, extensively used to study the epidermal homeostasis and pathophysiology, were analysed for the expression of the three known MV receptors: CD150, CD46 and nectin-4 by flow cytometry. While both primary keratinocytes and HaCaT express nectin-4 and CD46, the expression of CD150 was not detected (Fig. 1).

Then, we analysed the susceptibility of human keratinocytes to different MV strains expressing either vaccine or wt MV envelope glycoprotein H, responsible for the differential receptor binding. To follow infection daily by immunofluorescence, we used recombinant viruses expressing EGFP: Schwarz-EGFP (rSchwarz), made from the vaccine Schwarz strain, IC323-EGFP-Hwt (IC323-Hwt) produced from the wt IC323 strain and IC323-EGFP-Hvac (IC323-Hvac) made from the wt IC323 strain bearing the replacement of the wt H gene with Schwarz vaccine H gene. While MV strains expressing a vaccine H protein, interact with CD46, CD150 and nectin-4 receptors, MV strain expressing wt H protein, can interact only with CD150 and nectin-4 receptors. All analysed MV strains infected keratinocytes and induced cytopathic effects, as evidenced by the syncytia formation (Fig. 2**a** and **b**). Interestingly, the production of infectious viral particles from infected primary keratinocytes was much higher following the infection with IC323-Hvac, then with IC323-Hwt (Fig. 2**c**), possibly resulting from the utilization of both CD46 and nectin-4 receptors by IC323-Hvac for the virus entry, in contrast to the utilisation of only nectin-4 for IC323-Hwt.

3.2. MV infection modulates cytokine expression in human keratinocytes

We next investigated the effect of MV infection on cytokine expression in keratinocytes. HaCaT were infected with MV Schwarz, contained in ROUVAX vaccine, or incubated with the UV-irradiated virus and analysed at 6 h and 24 h, when cell layer

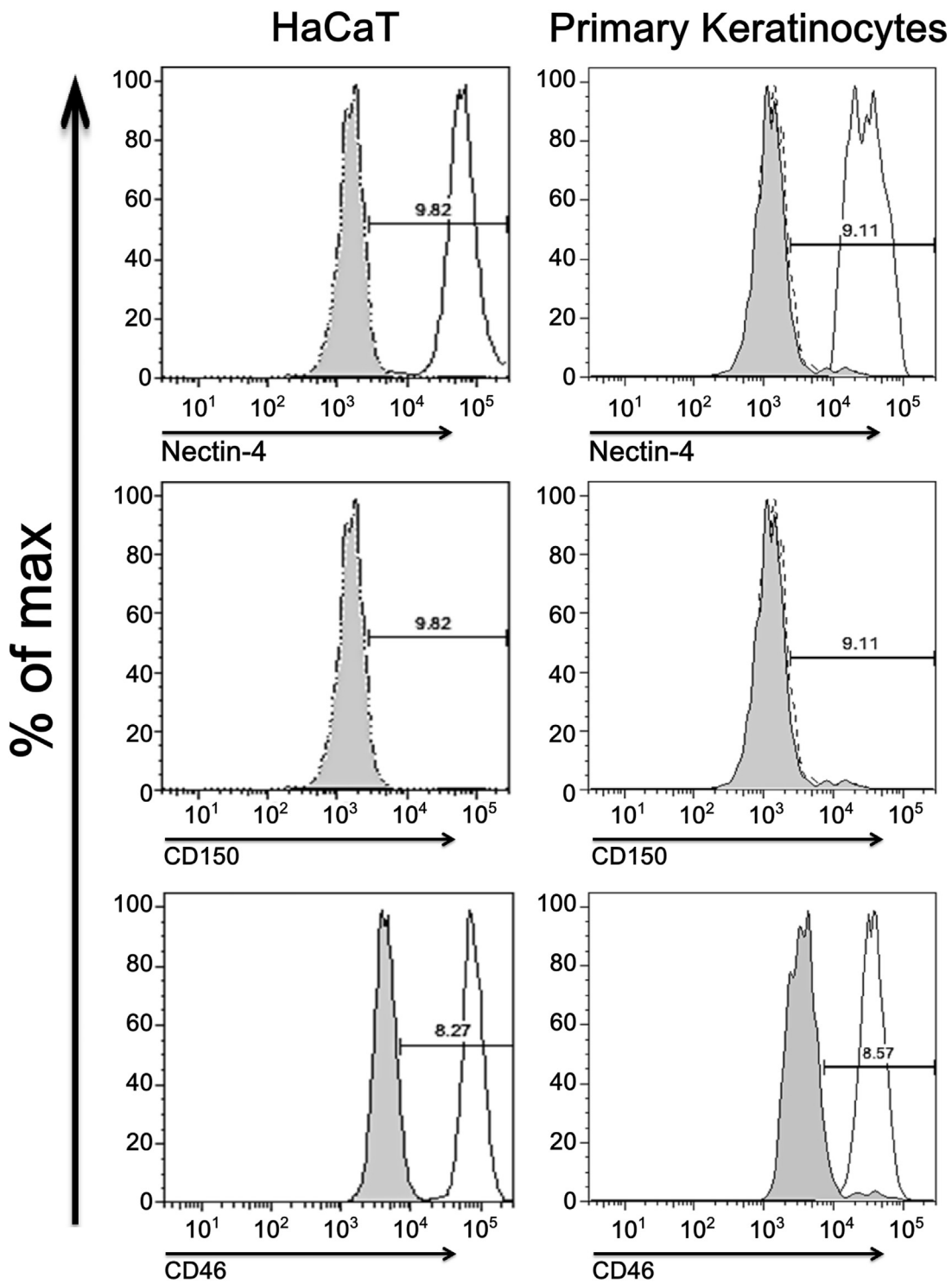

Fig 1. Expression of MV receptors by keratinocytes. The keratinocyte cell line HaCaT and primary keratinocytes were analysed for the expression of the three known MV receptors: nectin-4, CD150 and CD46 by flow cytometry. Filled histograms: non-stained cells, open histogram stained cells. Data from one representative experiment out of 5 performed are presented. Values indicate the percentage of positive cells.

integrity was still preserved. We measured in infected HaCaT cells by RT-qPCR the expression level of cytokines TSLP, CCL26 and TGFβ, all known to be produced by keratinocytes and involved in the pathogenesis of AD. While cytokine profiles remain unchanged at 6 h post-infection (p.i.), mRNA levels of TSLP and CCL26 were significantly decreased at 24 h p.i., only when keratinocytes were exposed to the infectious but not to the UV-treated MV. Finally, in contrast to the decreased level of TSLP and CCL26, the mRNA level of TGF-β was significantly increased in MV-infected keratinocytes 24 h p.i. (Fig. 3).

3.3. Effect of MV vaccination on the skin cellular immune response in AD patients

As above *in vitro* studies suggested that in keratinocytes MV vaccine strain could downregulate the production of cytokines

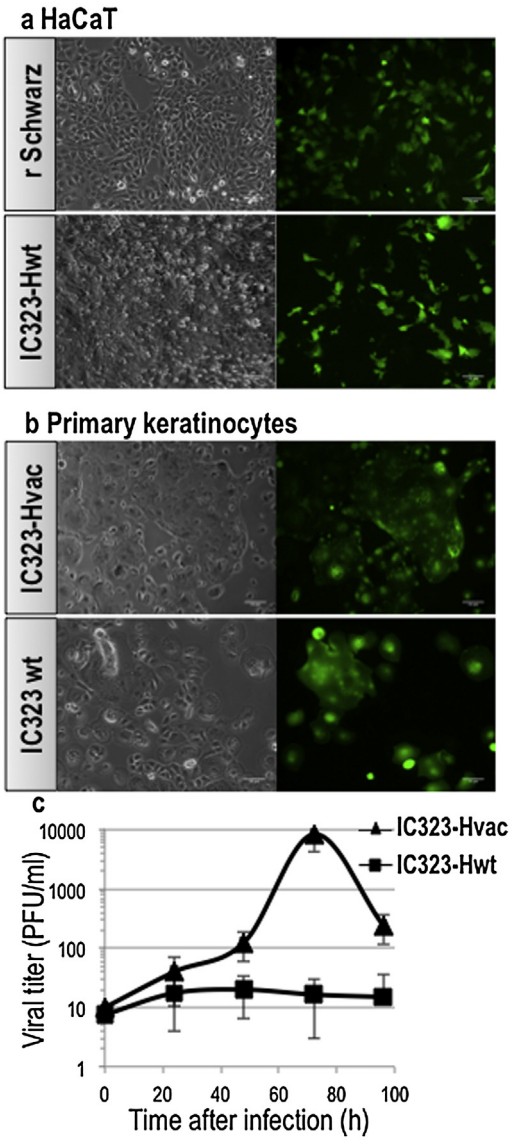

Fig. 2. MV infection of keratinocytes. Phase contrast (on the left) and fluorescence image (on the right) of MV infected HaCaT keratinocytes (**a**) and primary human keratinocytes (**b**) 72 h post infection (MOI = 1), using either virus expressing a vaccine or wt H protein: recombinant (r) Schwarz and IC323-vac or IC323-Hwt, respectively. Scale bar: 20 μm. (**c**) Production of infectious viral particles from primary human keratinocytes, infected at MOI = 1 with either IC323-Hvac or IC323-Hwt. Viral titers were determined by plaque forming assay at different times post-infection.

known to play an important role in the AD pathogenesis, we next performed a clinical assay to explore the potential effect of MV vaccine in AD *in vivo*. The study included 20 adult patients with moderate AD SCORAD, receiving either MV vaccine ROUVAX or placebo.

As MV infection and vaccination were described to be associated with a decreased delayed-type hypersensitivity reaction (DTH) [33,21], we analysed DTH response to tuberculin, using the classical PPD sensitization. In addition, to extend the analysis of cellular immune response to the other antigens, we performed an atopy patch test using 7 different allergens. The mean score of atopy patch test was slightly decreased in patients after vaccination (Fig. 4**a**), but the statistical difference was not observed between the groups (Fig. 4).

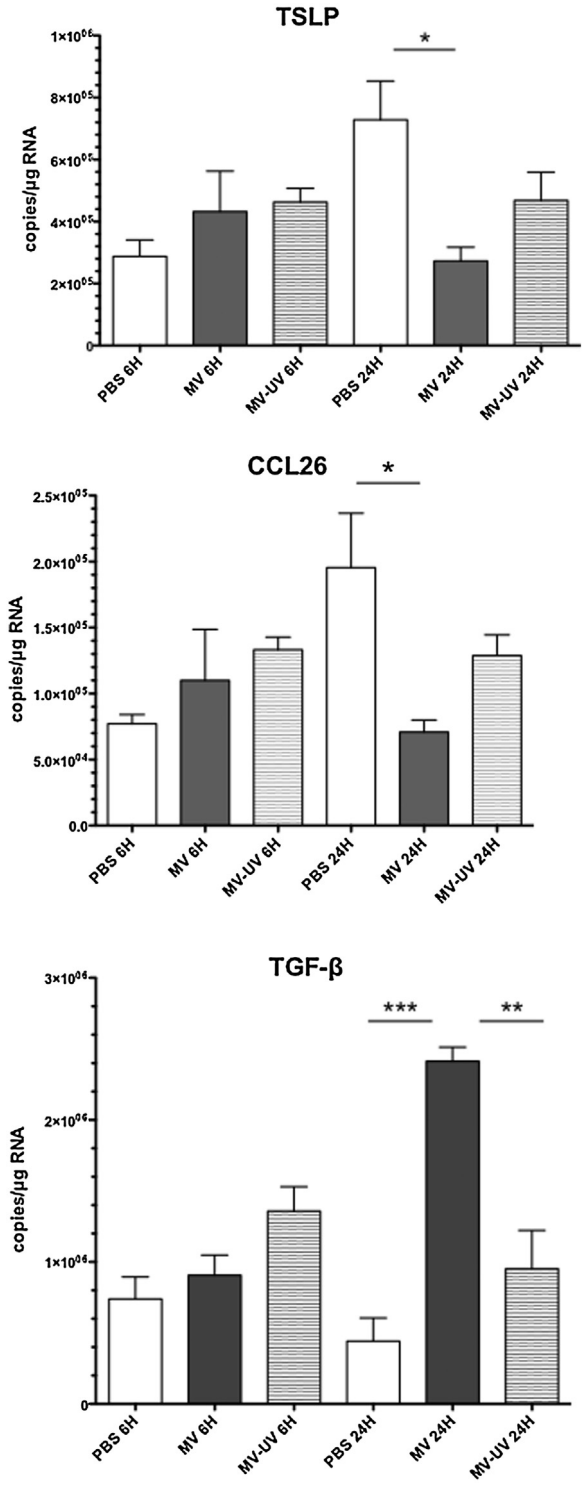

Fig. 3. MV-induced expression of cytokines in human keratinocytes. HaCaT cells were infected with MV Schwarz (Rouvax, MOI = 1) or incubated with the same amount of UV-inactivated MV or with PBS and analysed for the expression of TSLP, CCL26 and TGF-β at 6 h and 24 h post-infection, by RT-qPCR. Results are expressed as a number of copies/μg of RNA extracted from indicated samples, with histograms presenting mean values ± SD from two experiments. Statistical differences were determined using ANOVA followed by a Bonferroni's post hoc multiple comparison test ($^{*}P < 0{,}05$, $^{**}P > 0.01$, $^{***}P < 0{,}001$).

3.4. Effect of MV vaccination on the cytokine profile of AD skin

To determine whether MV vaccination could modulate cytokine production in the human skin, similarly to what we observed in

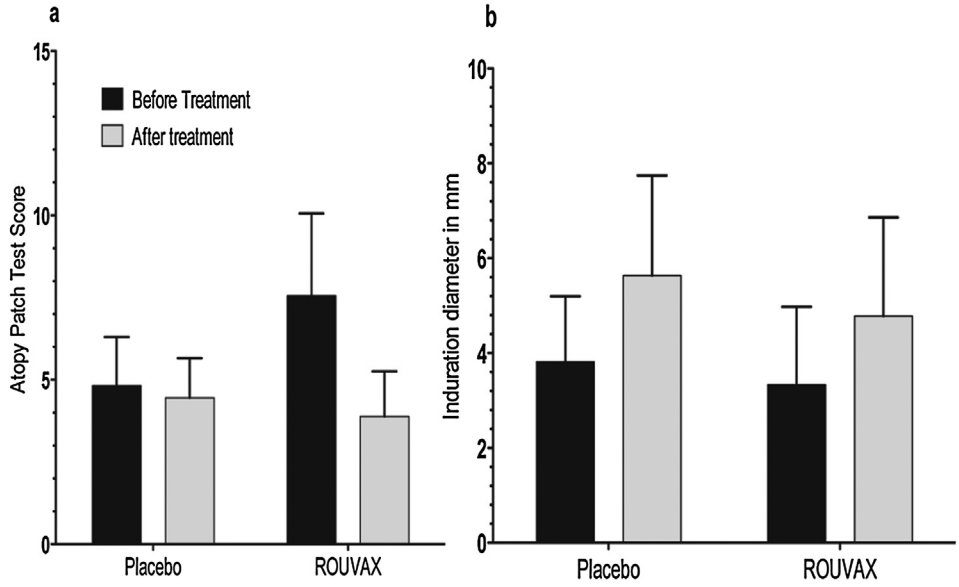

Fig. 4. Evaluation of atopy patch test reactivity and tuberculin sensitivity in AD patients. Atopy path and tuberculin sensitivity tests were performed 3 days before the treatment and 7 days after the treatment, which consisted of either ROUVAX (n = 9) or placebo (n = 11) injections. Histograms present the average score ± SD for atopy patch test, determined from the sum of scores obtained for individual allergens for each patient (**a**) and the average induration diameter (mm) ± SD for tuberculin test (**b**).

keratinocytes *in vitro*, we analysed the expression of TSLP, CCL26 and TGF-β, as well as several other cytokines involved in the pathogenesis of AD: CCL11, CCL17/TARK and CCL27, in skin biopsies of AD patients involved in the clinical trial. Biopsies were obtained either from clinically non-involved skin regions or skin lesions after exposure to atopy patch test in AD patients vaccinated with ROUVAX or not (placebo). Although no differences were observed on day 0, levels of TSLP and CCL26 mRNA were significantly lower in skin lesions obtained 10 days after MV vaccination than in the placebo group (Fig. 5), suggesting a decrease in the production of those cytokines in the skin of vaccinated AD patients. Finally, no changes were observed in the TGF-β mRNA expression (Fig. 5), neither in the expression of CCL11, CCL17 and CCL27 (data not shown).

3.5. Evolution of SCORAD after MV vaccination

To determine whether ROUVAX vaccination may have a clinical impact on the progression of AD, patients were followed throughout the study by the evaluation of the clinical severity score of AD, by SCORAD (Fig. 6). Although the SCORAD was not statistically different between vaccinated and placebo-treated patients at the beginning (day 0) and at the end of study (day 42), a moderate but statistically significant, difference was observed 2 weeks after vaccination. Induction of MV-specific IgG antibodies was observed in the serum of patients 3 weeks after vaccination (Supplementary Fig. 1) However, the evolution of SCORAD did not correlate with the change in serum level of MV-specific antibodies in patients following vaccination (data not shown). Altogether, these results suggest some transient improvement of clinical severity of AD after MV vaccination, which will need to be further confirmed on higher number of patients.

4. Discussion

Keratinocytes have a key role in innate immunity and the detection of pathogens, contributing to host defence [34], through their expression of many pattern-recognition receptors and the production of a variety of cytokines in response to pathogenic stimuli [35,36]. Although MV infection is marked with the skin rash in the acute phase of the disease, MV infection of keratinocytes has been neglected, mainly due to the known absence of the MV receptor CD150 expression in this cell type. However, the recent discovery of nectin-4 as an additional MV receptor [12,13] and its expression in skin [15] renewed the interest in analysing the importance of this cell population in MV pathogenesis. Our study clearly demonstrated that both the human keratinocyte cell line HaCaT, and primary keratinocytes are susceptible to the infection with both wt and vaccine MV strains. These results are in agreement with recent studies showing the expression of MV antigens in epithelial cells and skin of monkeys infected either with wt [37,38] or vaccine MV [39,40]. Furthermore, another morbillivirus close to MV, the canine distemper virus, was reported to infect primary canine keratinocytes [41]. Our study has demonstrated that infection of primary keratinocytes with wt MV resulted only in a low level of production of infectious virus. Similar results were obtained using human epithelial cells [42], suggesting that, in contrast to lymphoid cells, the infection of epithelial cells may have a limited role in MV propagation within the organism. However, the effect of MV infection on the biology of those cells has not been studied to date.

Cutaneous homeostasis and anti-microbial defence is maintained by a permanent cross-talk between keratinocytes and the cells of the immune system, through the production of cytokines, directly implicated in the control of skin barrier [43]. Our results have demonstrated that MV infection in keratinocytes *in vitro* decreases the expression of the proinflammatory cytokines TSLP and CCL26, and in parallel, strongly increases the immunosuppressive TGF-β. TSLP has recently been implicated as a key molecule for initiating allergic inflammation at the epithelial cell-dendritic cell interface [44] and is highly expressed in keratinocytes from patients with AD [45]. CCL26 is involved in the migration of cells expressing CCR3 in AD [46]. In addition to the decreased expression of TSLP and CCL26 after *in vitro* MV infection of keratinocytes, we also found their reduced expression in biopsies of lesional skin from AD patients after MV vaccination. Together, these findings suggest that the MV-induced modulation of cytokine expression in the skin may participate in the control of

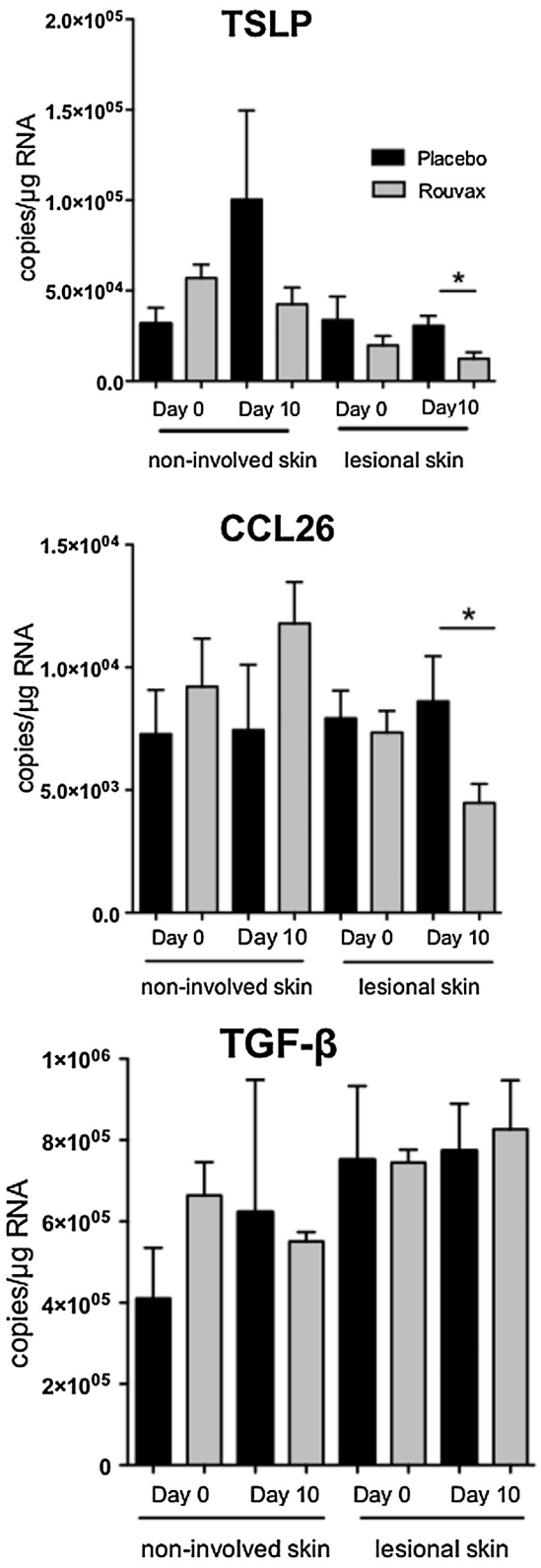

Fig. 5. Effect of MV vaccination on cytokine gene expression in the skin of AD patients. Biopsies were taken before vaccination (day 0) and 10 days later, from either clinically non-involved skin (without visible AD lesions) or from the acute skin lesions following the atopy patch test (n = 3–7). RNA were extracted and analysed for the expression of TSLP, CCL26 and TGF-β by RT-qPCR. Results are expressed as a number of copies/µg of RNA extracted from indicated samples, with histograms presenting mean values ± SD. $^{*}P < 0,05$, ANOVA followed by a Bonferroni's post hoc multiple comparison test.

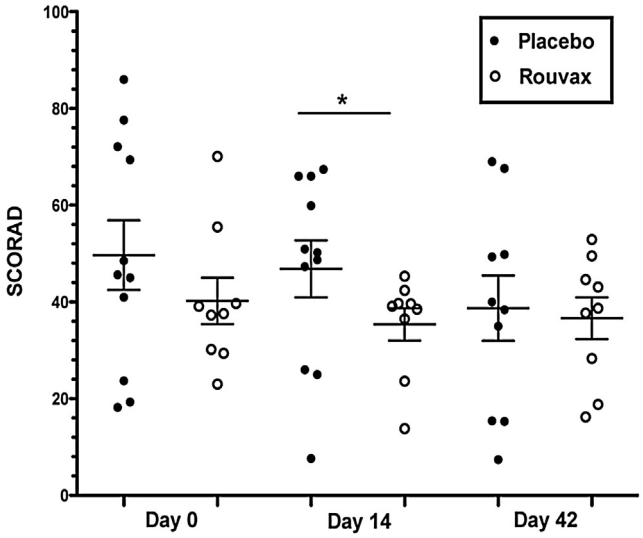

Fig. 6. Changes in the clinical scores (SCORAD) of AD patients. After receiving either ROUVAX (n = 9 patients) or placebo treatment (n = 11), patients were followed during six weeks. Histograms present mean values ± SD. $^{*}P < 0,05$, Mann-Whitney rank sum test.

AD pathogenesis. As the change of cytokine profile was found only in acute skin lesions and not in non-involved skin, it may be possible that MV infection does not influence the steady state cytokine expression, but might affect it following an exposure to allergens.

The results obtained in this study have shown a transient improvement of the clinical score of AD patients, two weeks after MV vaccination. Beneficial effect of MV vaccination on atopic sensitization was previously observed in the big cohort of children attending Steiner schools in Europe [47], and measles immunization was suggested to have a nonspecific positive effect in the reduction of mortality in developing countries [48]. Interestingly, a decrease in mitogen-induced proliferation of lymphoid cells in infants was also shown two weeks after measles vaccination [22], suggesting that this period post-vaccination may be optimal to observe a systemic effect of the vaccine on the immune response. In agreement with these findings, our previous study has shown that MV immunization in infants does not aggravate AD and may improve some immunologic parameters, including decrease in the serum level of CCL18, chemokine associated with the severity of AD [49]. In this study the inhibition of the TSLP and CCL26 expression in the skin biopsies in AD patients 10 days after MV vaccination, suggests the potential link between decrease in the production of these highly proinflammatory cytokines and the improvement of clinical symptoms in AD patients. However, more detailed mechanisms involved in MV-induced modulation of AD remain to be elucidated.

We have previously shown that MV proteins could inhibit skin hypersensitivity response in mice by engagement of CD46 and through Fcγ receptor on dendritic cells [50]. MV receptor CD46 was reported to be involved in the generation of regulatory T cells [51], a cell population important in AD pathogenesis [52]. Finally, the engagement of the second MV receptor, the human molecule CD150, was shown to induce the reversal of human allergic Th2-type lymphocytes, isolated from skin biopsies of AD patients, into a Th1 profile [53]. Taken together, these results suggest that different mechanisms may be implicated in the beneficial effect of anti-measles vaccination in allergic inflammatory diseases. This study provides an additional argument of its advantageous effect in AD, suggesting that MV infection may be directly responsible for

the down-modulation of skin keratinocyte proinflammatory profile, which could contribute to systemic effects of MV vaccination in the control of AD pathogenesis.

Despite the increased prevalence of AD in the last decades [16] and the extensive study of the disease, the events leading to AD development and pathogenesis are not fully understood and new therapeutic approaches are highly desirable. As live attenuated MV is widely utilized and well-tolerated, future research in this area and the identification of the immunomodulatory MV proteins holds promise for the development of the novel treatment strategies. The obtained results, by providing proof of concept for the vaccination therapy in AD, may initiate a development of new therapeutic approaches, innovative and inexpensive and potentially efficient in the treatment of not only AD, but also the other allergic inflammatory diseases.

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Disclosures

The authors have no conflict of interest to declare

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jdermsci.2017.01.015.

References

[1] D. Butler, Measles by the numbers: a race to eradication, Nature 518 (2015) 148–149.
[2] D.E. Griffin, W.-H. Lin, C.-H. Pan, Measles virus immune control, and persistence, FEMS Microbiol. Rev. 36 (2012) 649–662.
[3] T.R. Moench, D.E. Griffin, C.R. Obriecht, A.J. Vaisberg, R.T. Johnson, Acute measles in patients with and without neurological involvement: distribution of measles virus antigen and RNA, J. Infect. Dis. 158 (1988) 433–442.
[4] A. Kimura, K. Tosaka, T. Nakao, An immunofluorescent and electron microscopic study of measles skin eruptions, Tohoku J. Exp. Med. 117 (1975) 245–256.
[5] A. Kimura, K. Tosaka, T. Nakao, Measles rash. I. Light and electron microscopic study of skin eruptions, Arch. Virol. 47 (1975) 295–307.
[6] H. Takahashi, Y. Umino, T.A. Sato, T. Kohama, Y. Ikeda, M. Iijima, R. Fujisawa, Detection and comparison of viral antigens in measles and rubella rashes, Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am. 22 (1996) 36–39.
[7] E. Olding-Stenkvist, B. Bjorvatn, Rapid detection of measles virus in skin rashes by immunofluroescence, J. Infect. Dis. 134 (1976) 463–469.
[8] D.W. Suringa, L.J. Bank, A.B. Ackerman, Role of measles virus in skin lesions and Koplik's spots, N. Engl. J. Med. 283 (1970) 1139–1142.
[9] D. Naniche, G. Varior-Krishnan, F. Cervoni, T.F. Wild, B. Rossi, C. Rabourdin-Combe, D. Gerlier, Human membrane cofactor protein (CD46) acts as a cellular receptor for measles virus, J. Virol. 67 (1993) 6025–6032.
[10] R.E. Dorig, A. Marcil, A. Chopra, C.D. Richardson, The human CD46 molecule is a receptor for measles virus (Edmonston strain), Cell 75 (1993) 295–305.
[11] H. Tatsuo, N. Ono, K. Tanaka, Y. Yanagi, SLAM (CDw150) is a cellular receptor for measles virus, Nature 406 (2000) 893–897.
[12] M.D. Muhlebach, M. Mateo, P.L. Sinn, S. Prufer, K.M. Uhlig, V.H. Leonard, C.K. Navaratnarajah, M. Frenzke, X.X. Wong, B. Sawatsky, S. Ramachandran, P.B. McCray Jr., K. Cichutek, V. von Messling, M. Lopez, R. Cattaneo, Adherens junction protein nectin-4 is the epithelial receptor for measles virus, Nature 480 (2011) 530–533.
[13] R.S. Noyce, D.G. Bondre, M.N. Ha, L.T. Lin, G. Sisson, M.S. Tsao, C.D. Richardson, Tumor cell marker PVRL4 (nectin 4) is an epithelial cell receptor for measles virus, PLoS Pathog. 7 (2011).
[14] S.P. Sidorenko, E.A. Clark, The dual-function CD150 receptor subfamily: the viral attraction, Nat. Immunol. 4 (2003) 19–24.
[15] F. Brancati, P. Fortugno, I. Bottillo, M. Lopez, E. Josselin, O. Boudghene-Stambouli, E. Agolini, L. Bernardini, E. Bellacchio, M. Iannicelli, A. Rossi, A. Dib-Lachachi, L. Stuppia, G. Palka, S. Mundlos, S. Stricker, U. Kornak, G. Zambruno, B. Dallapiccola, Mutations in PVRL4 encoding cell adhesion molecule nectin-4, cause ectodermal dysplasia-syndactyly syndrome, Am. J. Hum. Genet. 87 (2010) 265–273.
[16] D.Y. Leung, M. Boguniewicz, M.D. Howell, I. Nomura, Q.A. Hamid, New insights into atopic dermatitis, J. Clin. Invest. 113 (2004) 651–657.
[17] A.B. Olesen, S. Juul, K. Thestrup-Pedersen, Atopic dermatitis is increased following vaccination for measles, mumps and rubella or measles infection, Acta Derm. Venereol. 83 (2003) 445–450.
[18] C. Bodner, W.J. Anderson, T.S. Reid, D.J. Godden, Childhood exposure to infection and risk of adult onset wheeze and atopy, Thorax 55 (2000) 383–387.
[19] A.L. Boner, E.A. Valletta, J.A. Bellanti, Improvement of atopic dermatitis following natural measles virus infection. Four case reports, Ann. Allergy 55 (1985) 605–608.
[20] S.O. Shaheen, P. Aaby, A.J. Hall, D.J. Barker, C.B. Heyes, A.W. Shiell, A. Goudiaby, Measles and atopy in Guinea-Bissau, Lancet 347 (1996) 1792–1796.
[21] P. Fireman, G. Friday, J. Kumate, Effect of measles vaccine on immunologic responsiveness, Pediatrics 43 (1969) 264–272.
[22] G.D. Hussey, E.A. Goddard, J. Hughes, J.J. Ryon, M. Kerran, E. Carelse, P.M. Strebel, L.E. Markowitz, J. Moodie, P. Barron, Z. Latief, R. Sayed, D. Beatty, D.E. Griffin, The effect of Edmonston-Zagreb and Schwarz measles vaccines on immune response in infants, J. Infect. Dis. 173 (1996) 1320–1326.
[23] H.F. Pabst, D.W. Spady, M.M. Carson, H.T. Stelfox, J.A. Beeler, M.P. Krezolek, Kinetics of immunologic responses after primary MMR vaccination, Vaccine 15 (1997) 10–14.
[24] R.L. Hirsch, F. Mokhtarian, D.E. Griffin, B.R. Brooks, J. Hess, R.T. Johnson, Measles virus vaccination of measles seropositive individuals suppresses lymphocyte proliferation and chemotactic factor production, Clin. Immunol. Immunopathol. 21 (1981) 341–350.
[25] P. Boukamp, R.T. Petrussevska, D. Breitkreutz, J. Hornung, A. Markham, N.E. Fusenig, Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line, J. Cell Biol. 106 (1988) 761–771.
[26] F. Radecke, P. Spielhofer, H. Schneider, K. Kaelin, M. Huber, C. Dotsch, G. Christiansen, M.A. Billeter, Rescue of measles viruses from cloned DNA, EMBO J. 14 (1995) 5773–5784.
[27] K. Boniface, F.-X. Bernard, M. Garcia, A.L. Gurney, J.-C. Lecron, F. Morel, IL-22 inhibits epidermal differentiation and induces proinflammatory gene expression and migration of human keratinocytes, J. Immunol. Baltim. Md 1950 174 (2005) 3695–3702.
[28] K. Hashimoto, N. Ono, H. Tatsuo, H. Minagawa, M. Takeda, K. Takeuchi, Y. Yanagi, SLAM (CD150)-independent measles virus entry as revealed by recombinant virus expressing green fluorescent protein, J. Virol. 76 (2002) 6743–6749.
[29] P. Devaux, V. von Messling, W. Songsungthong, C. Springfeld, R. Cattaneo, Tyrosine 110 in the measles virus phosphoprotein is required to block STAT1 phosphorylation, Virology 360 (2007) 72–83.
[30] B. Kunz, A. Oranje, L. Labreze, J. Stalder, J. Ring, A. Taieb, Clinical validation and guidelines for the SCORAD index: consensus report of the European task force on atopic dermatitis, Dermatology 195 (1997) 10–19.
[31] C. Goujon, C. Jean-Decoster, K. Dahel, D. Bottigioli, F. Lahbari, J.-F. Nicolas, A.-M. Schmitt, Tolerance of oat-based topical products in cereal-sensitized adults with atopic dermatitis, Dermatol. Basel Switz. 218 (2009) 327–333.
[32] C. Mathieu, V. Guillaume, A. Sabine, K.C. Ong, K.T. Wong, C. Legras-Lachuer, B. Horvat, Lethal Nipah virus infection induces rapid overexpression of CXCL10, PLoS One (2012) e32157.
[33] V.G. Tamashiro, H.H. Perez, D.E. Griffin, Prospective study of the magnitude and duration of changes in tuberculin reactivity during uncomplicated and complicated measles, Pediatr. Infect. J. 6 (1987) 451–454.
[34] I.-H. Kuo, T. Yoshida, A. De Benedetto, L.A. Beck, The cutaneous innate immune response in patients with atopic dermatitis, J. Allergy Clin. Immunol. 131 (2013) 266–278.
[35] T. Kawamura, Y. Ogawa, R. Aoki, S. Shimada, Innate and intrinsic antiviral immunity in skin, J. Dermatol. Sci. 75 (2014) 159–166, doi:http://dx.doi.org/10.1016/j.jdermsci.2014.05.004.
[36] F.O. Nestle, P. Di Meglio, J.-Z. Qin, B.J. Nickoloff, Skin immune sentinels in health and disease, Nat. Rev. Immunol. 9 (2009) 679–691.

[37] M. Ludlow, K. Lemon, R.D. de Vries, S. McQuaid, E.L. Millar, G. van Amerongen, S. Yüksel, R.J. Verburgh, A.D.M.E. Osterhaus, R.L. de Swart, W.P. Duprex, Measles virus infection of epithelial cells in the macaque upper respiratory tract is mediated by subepithelial immune cells, J. Virol. 87 (2013) 4033–4042.
[38] R.L. de Swart, M. Ludlow, L. de Witte, Y. Yanagi, G. van Amerongen, S. McQuaid, S. Yuksel, T.B. Geijtenbeek, W.P. Duprex, A.D. Osterhaus, Predominant infection of CD150+ lymphocytes and dendritic cells during measles virus infection of macaques, PLoS Pathog. 3 (2007).
[39] L.J. Rennick, R.D. de Vries, T.J. Carsillo, K. Lemon, G. van Amerongen, M. Ludlow, D.T. Nguyen, S. Yüksel, R.J. Verburgh, P. Haddock, S. McQuaid, W.P. Duprex, R.L. de Swart, Live-attenuated measles virus vaccine targets dendritic cells and macrophages in muscle of nonhuman primates, J. Virol. 89 (2015) 2192–2200.
[40] K. Takeuchi, N. Nagata, S.-I. Kato, Y. Ami, Y. Suzaki, T. Suzuki, Y. Sato, Y. Tsunetsugu-Yokota, K. Mori, N. Van Nguyen, H. Kimura, K. Nagata, Wild-type measles virus with the hemagglutinin protein of the edmonston vaccine strain retains wild-type tropism in macaques, J. Virol. 86 (2012) 3027–3037.
[41] J.P.M. Langedijk, J. Janda, F.C. Origgi, C. Örvell, M. Vandevelde, A. Zurbriggen, P. Plattet, Canine distemper virus infects canine keratinocytes and immune cells by using overlapping and distinct regions located on one side of the attachment protein, J. Virol. 85 (2011) 11242–11254.
[42] M. Ludlow, L.J. Rennick, S. Sarlang, G. Skibinski, S. McQuaid, T. Moore, R.L. de Swart, W.P. Duprex, Wild-type measles virus infection of primary epithelial cells occurs via the basolateral surface without syncytium formation or release of infectious virus, J. Gen. Virol. 91 (2010) 971–979.
[43] F.-X. Bernard, F. Morel, M. Camus, N. Pedretti, C. Barrault, J. Garnier, J.-C. Lecron, Keratinocytes under fire of proinflammatory cytokines: bona fide innate immune cells involved in the physiopathology of chronic atopic dermatitis and psoriasis, J. Allergy 2012 (2012) 718725.
[44] T. Ito, Y.-J. Liu, K. Arima, Cellular and molecular mechanisms of TSLP function in human allergic disorders–TSLP programs the Th2 code in dendritic cells, Allergol. Int. Off. J. Jpn. Soc. Allergol. 61 (2012) 35–43.
[45] V. Soumelis, P.A. Reche, H. Kanzler, W. Yuan, G. Edward, B. Homey, M. Gilliet, S. Ho, S. Antonenko, A. Lauerma, K. Smith, D. Gorman, S. Zurawski, J. Abrams, S. Menon, T. McClanahan, R. de Waal-Malefyt Rd, F. Bazan, R.A. Kastelein, Y.-J. Liu, Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP, Nat. Immunol. 3 (2002) 673–680.
[46] M. Sugaya, Chemokines and skin diseases, Arch. Immunol. Ther. Exp. (Warsz.) 63 (2015) 109–115.
[47] H. Rosenlund, A. Bergström, J.S. Alm, J. Swartz, A. Scheynius, M. van Hage, K. Johansen, B. Brunekreef, E. von Mutius, M.J. Ege, J. Riedler, C. Braun-Fahrländer, M. Waser, G. Pershagen, PARSIFAL study group, allergic disease and atopic sensitization in children in relation to measles vaccination and measles infection, Pediatrics 123 (2009) 771–778.
[48] P. Aaby, A. Bhuiya, L. Nahar, K. Knudsen, A. de Francisco, M. Strong, The survival benefit of measles immunization may not be explained entirely by the prevention of measles disease: a community study from rural Bangladesh, Int. J. Epidemiol. 32 (2003) 106–116.
[49] A. Hennino, C. Cornu, A. Rozieres, F. Augey, F. Villard-Truc, F. Payot, A. Lachaux, J.F. Nicolas, B. Horvat, Influence of measles vaccination on the progression of atopic dermatitis in infants, Pediatr. Allergy Immunol. 18 (2007) 385–390.
[50] J.C. Marie, J. Kehren, M.C. Trescol-Biemont, A. Evlashev, H. Valentin, T. Walzer, R. Tedone, B. Loveland, J.F. Nicolas, C. Rabourdin-Combe, B. Horvat, Mechanism of measles virus-induced suppression of inflammatory immune responses, Immunity 14 (2001) 69–79.
[51] C. Kemper, A.C. Chan, J.M. Green, K.A. Brett, K.M. Murphy, J.P. Atkinson, Activation of human CD4+ cells with CD3 and CD46 induces a T-regulatory cell 1 phenotype, Nature 421 (2003) 388–392.
[52] L.S. Ou, E. Goleva, C. Hall, D.Y. Leung, T regulatory cells in atopic dermatitis and subversion of their activity by superantigens, J. Allergy Clin. Immunol. 113 (2004) 756–763.
[53] J.M. Carballido, G. Aversa, K. Kaltoft, B.G. Cocks, J. Punnonen, H. Yssel, K. Thestrup-Pedersen, J.E. de Vries, Reversal of human allergic T helper 2 responses by engagement of signaling lymphocytic activation molecule, J. Immunol. 159 (1997) 4316–4321.