Journal of Dermatological Science xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Journal of Dermatological Science

journal homepage: www.jdsjournal.com

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

ARTICLE INFO

Article history:
Received 10 November 2016
Received in revised form 21 December 2016
Accepted 10 January 2017

Keywords:
Measles virus
Keratinocytes
Cytokines
Vaccination
Atopic dermatitis
Immune response

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).

http://dx.doi.org/10.1016/j.jdermsci.2017.01.015
0923-1811/

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.

Please cite this article in press as: G. Gourru-Lesimple, et al., Measles virus infection of human keratinocytes: Possible link between measles and atopic dermatitis, J Dermatol Sci (2017), http://dx.doi.org/10.1016/j.jdermsci.2017.01.015