RMD Open
Rheumatic & Musculoskeletal Diseases

CLINICAL CASE

Dermatomyositis flare on imiquimod therapy highlights a crucial role of aberrant TLR7 signalling

Alain Meyer,[1,2,3] Ghada Alsaleh,[3,4] Claude Heuschling,[5] Jacques-Eric Gottenberg,[2,3] Philippe Georgel,[3,4] Benard Geny,[2,3] Seiamak Bahram,[3,4] Jean Sibilia[2,3,4]

To cite: Meyer A, Alsaleh G, Heuschling C, *et al*. Dermatomyositis flare on imiquimod therapy highlights a crucial role of aberrant TLR7 signalling. *RMD Open* 2016;**2**:e000294. doi:10.1136/rmdopen-2016-000294

▸ Prepublication history for this paper is available online. To view these files please visit the journal online (http://dx.doi.org/10.1136/rmdopen-2016-000294).

AM and GA contributed equally.

Received 10 April 2016
Revised 26 August 2016
Accepted 29 September 2016

For numbered affiliations see end of article.

Correspondence to
Dr Alain Meyer;
alain.meyer1@chru-strasbourg.fr

Dermatomyositis (DM) is a chronic systemic disease that primarily affects skin and/or muscles and is associated with cancer in about 20% of cases. Although DM is an autoimmune disorder, there is evidence that innate immunity plays a crucial role in the disease. In particular, it has been associated with elevated interferon (IFN)-β in blood[1] which is critical in the initiation[2] and perpetuation[3] of the disease. However, the origin of elevated IFN-β remains elusive. It has been speculated that it may result from the engagement of endosomal toll-like receptor (TLR) signalling due to the increased expressions of TLR7 and TLR9 in peripheral blood leucocytes[4] of patients with DM, but direct evidence of endosomal TLR involvement in DM is lacking.

We report herein a patient who developed severe exacerbation of anti-NXP2-positive DM on imiquimod therapy, a potent TLR7 agonist approved for the treatment of cutaneous basocellular carcinoma. Peripheral blood mononuclear cells (PBMC) analysis revealed an increase in IFN-β secretion on TLR7 stimulation, whereas TLR4-induced pro-inflammatory cytokines secretion did not differ from healthy matched controls. This report not only provides evidence that endosomal TLR7 participates in human DM but also pointed to the skin as a primary organ allowing TLR7 agonists to induce DM flare.

A woman aged 47 years presented with fever, arthralgia, myalgia and a facial DM rash (figure 1). She declared that a slight facial rash suggesting of DM had been present for over 1 year and that its exacerbation and extracutaneous signs onset appeared 1 month after starting topic imiquimod (5%, once daily) for a cutaneous basocellular carcinoma of the anterior chest wall (diagnosed 6 months after DM rash onset). Temperature was 38°C, and limb girdle muscles were painful but with no signs of weakness. Joints of the hand were tender without arthritis. C reactive protein level was 1.5 mg/dL (normal <0.4) while creatine kinase level was normal. She tested positive

Key messages

What is already known about this subject?

- It has been speculated that elevated interferon (IFN)-β in blood characterising dermatomyositis patients results from the engagement of endosomal toll-like receptor (TLR) signalling due to the increased expressions of TLR7 and TLR9 in peripheral blood leucocytes.

What does this study add?

- We provide direct evidence that supports this hypothesis by reporting herein a patient who developed severe exacerbation of anti-NXP2-positive dermatomyositis (DM) on imiquimod therapy, a potent TLR7 agonist and whose peripheral blood mononuclear cells secreted high level of IFN-β upon TLR7 stimulation as compared with healthy donors.

How might this impact on clinical practice?

- These data indicate that aberrant TLR7 signalling may represent a therapeutic target in DM.

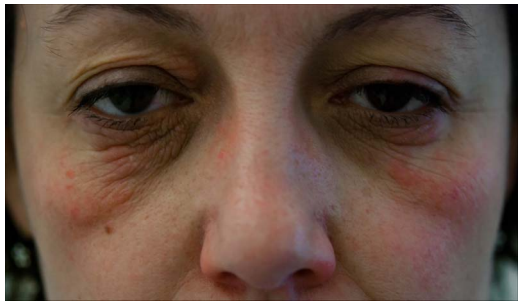

Figure 1 Eyelid erythema and oedema after imiquimod intake.

for anti-NXP2 antibodies (DTek and Euroimmun) and negative for anti-Mi2, anti-SAE, anti-TIF1γ and anti-MDA5. Electromyographic recordings were normal. Hand radiographs demonstrated no damage. CT and spirometry demonstrated no evidence of interstitial lung disease. Cutaneous basocellular carcinoma had hardly disappeared when she was diagnosed with DM and no other cancer was found on ^{18}F-FDG PET/CT, gastroscopy and colonoscopy. Eyelids skin rash, polyarthralgia, fever and anti-NXP2 demonstrated by two immunoassay test kits indicate that our patient did suffer from amyopathic DM. Accordingly, she was successfully treated with prednisone, topical tacrolimus for the DM rash and imiquimod discontinuation (after 6 weeks treatment and complete clinical regression of the basocellular carcinoma).

In vitro, TLR7 stimulation of the patient's PBMC (sampled 12 months after imiquimod discontinuation) led to an increase in several pro-inflammatory cytokines involved in DM,[5] including IFN-β. Imiquimod also lead to an increase in TLR7 expression in the PBMC of our

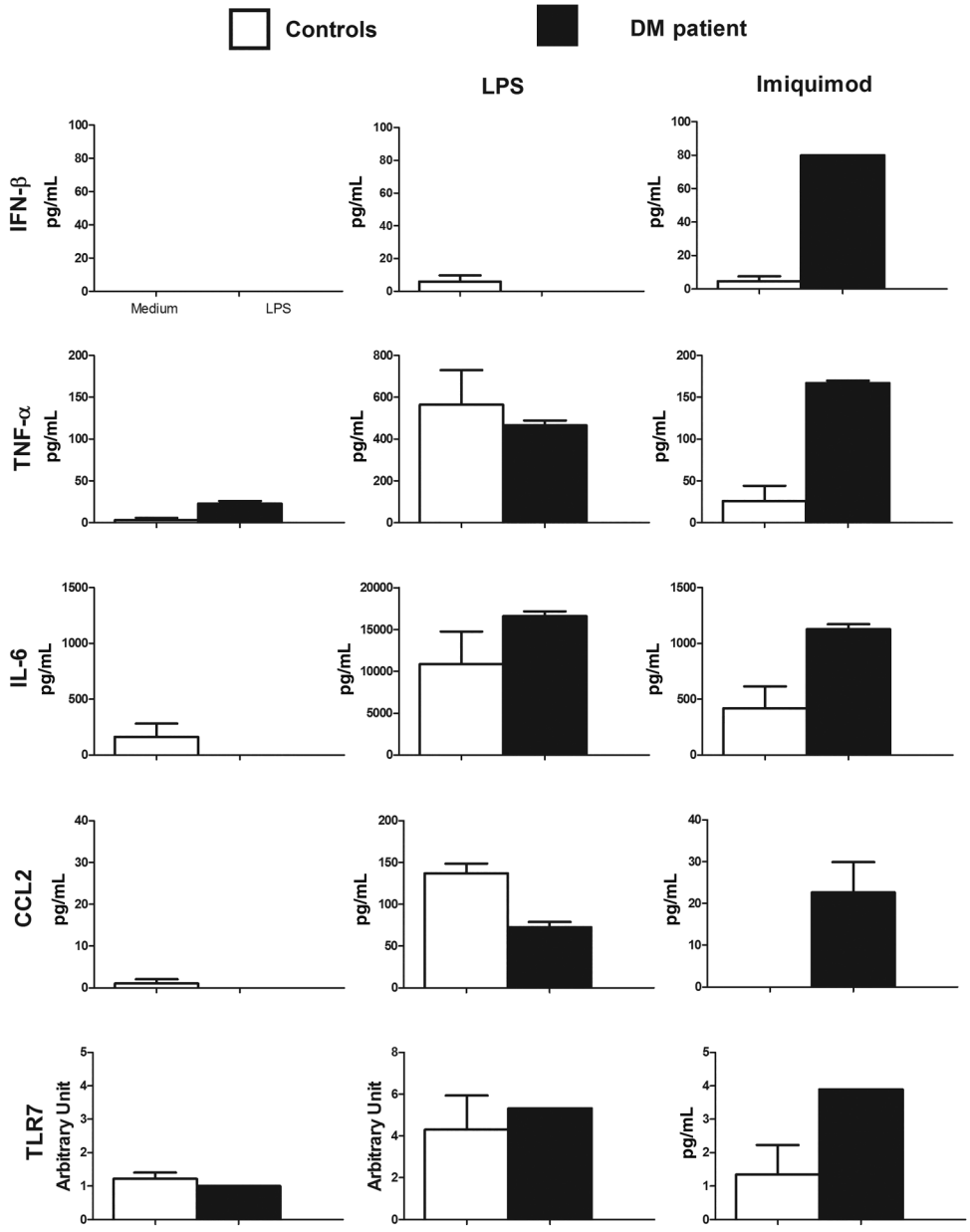

Figure 2 IFN-β, IL-6, TNF-α and CCL2 release were determined by ELISA in culture supernatants of PBMC stimulated with LPS from *Salmonella abortusequi* (1 µg/mL Sigma–Aldrich (Saint-Quentin-Fallavier, France)) or imiquimod (5 µg/mL, Sigma–Aldrich (Saint-Quentin-Fallavier, France)) for 3 hours. TLR7 expression was determined by RT-qPCR. Results were normalised to Gapdh and expressed as fold change compared with samples from cells incubated in medium alone. PBMC was isolated from the DM patient and three age-matched healthy controls. The patient had discontinued imiquimod 1-year topical tacrolimus 2 weeks before PBMC were sampled. CCL2, chemokine ligand 2; DM, dermatomyositis; IL-6, interleukin-6; INF-β, interferon-β; LPS, lipopolysaccharide; PBMC, peripheral blood mononuclear cells; TLR7, toll-like receptor-7; TNF-α, tumour necrosis factor-α.

patient but not in controls. Pro-inflammatory cytokine secretion on TLR4 stimulation, which expression has been reported to be unchanged in DM PBMC,[4] did not differ from age-matched female controls (figure 2).

The present report suggests that aberrant endosomal TLR signalling, including high IFN-β secretion by PBMC and TLR7 signal auto-amplification, participates in DM. Given the presence of a feed-forward loop between IFN-β and TLR7 signalling,[6] the latter is likely to participate in disease initiation and maintenance. This extends the previous description of abnormal endosomal TLR expression in PBMC of patients with DM.[4] Natural ligands of TLR7 in DM patients probably include microbial RNA since a clinical history consistent with an infection is frequently reported prior to disease onset,[7] but endogenous RNAs are also likely to be involved notably during cancer and UV radiation damages, two frequent local conditions involving TLR7-mediated response[8 9] and triggering DM. Consistently with this view, skin TLR7 activation in mice[10] and human[11] has been previously reported to trigger lupus, an autoimmune disease that is also characterised by a type on interferon signature in blood. These data indicate that TLR7 may represent a therapeutic target in DM.

Author affiliations
[1]Centre de Référence des Maladies Auto-immunes Rares, Hôpitaux Universitaires de Strasbourg, Strasbourg, France
[2]Nouvel Hôpital Civil, Service des Explorations Fonctionnelles, Hôpitaux Universitaires de Strasbourg, Strasbourg, France
[3]Fédération de Médecine Translationnelle (FMTS), Strasbourg, France
[4]Laboratoire d'ImmunoRhumatologie Moléculaire, INSERM UMR_S1109, Centre de Recherche d'Immunologie et d'Hématologie, Faculté de Médecine, Université de Strasbourg, Strasbourg, France
[5]Cabinet de Rhumatologie, Esch-sur-Alzette, Luxembourg

Acknowledgements The authors thank the patient and the volunteers who participated in this study.

Contributors AM, GA, J-EG, PG, BG, SB and JS made substantial contributions to conception and design. AM, GA, CH and JS made acquisition of data. AM, GA, J-EG, PG, BG, SB, JS made analysis and interpretation of data. AM, GA, CH, J-EG, PG, BG, SB and JS participated in drafting the article or revising it critically for important intellectual content. AM, GA, CH, J-EG, PG, BG, SB and JS gave final approval of the version to be submitted and any revised version.

Funding This work was funded by the Institut national de la santé et de la recherchemédicale (INSERM), the University of Strasbourg (UNISTRA) and the Institut Universitaire de France (IUF).

Competing interests None declared.

Patient consent Obtained.

Ethics approval Human cells were obtained after informed consent was obtained from donors. Ethical approval was granted by the institutional ethics committee, Hopitaux Universitaires de Strasbourg, France.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement Data reported in this manuscript will be shared if needed.

REFERENCES

1. Liao AP, Salajegheh M, Nazareno R, *et al*. Interferon β is associated with type 1 interferon-inducible gene expression in dermatomyositis. *Ann Rheum Dis* 2011;70:831–6.
2. Somani AK, Swick AR, Cooper KD, *et al*. Severe dermatomyositis triggered by interferon beta-1a therapy and associated with enhanced type I interferon signaling. *Arch Dermatol* 2008;144:1341–9.
3. Suárez-Calvet X, Gallardo E, Nogales-Gadea G, *et al*. Altered RIG-I/DDX58-mediated innate immunity in dermatomyositis. *J Pathol* 2014;233:258–68.
4. Sun WC, Sun YC, Lin H, *et al*. Dysregulation of the type I interferon system in adult-onset clinically amyopathic dermatomyositis has a potential contribution to the development of interstitial lung disease. *Br J Dermatol* 2012;167:1236–44.
5. De Paepe B, Creus KK, De Bleecker JL. Role of cytokines and chemokines in idiopathic inflammatory myopathies. *Curr Opin Rheumatol* 2009;21:610–16.
6. Green NM, Laws A, Kiefer K, *et al*. Murine B cell response to TLR7 ligands depends on an IFN-beta feedback loop. *J Immunol Baltim Md 1950* 2009;183:1569–76.
7. Manlhiot C, Liang L, Tran D, *et al*. Assessment of an infectious disease history preceding juvenile dermatomyositis symptom onset. *Rheumatol Oxf Engl* 2008;47:526–9.
8. Schön MP, Schön M. TLR7 and TLR8 as targets in cancer therapy. *Oncogene* 2008;27:190–9.
9. Fishelevich R, Zhao Y, Tuchinda P, *et al*. Imiquimod-induced TLR7 signaling enhances repair of DNA damage induced by ultraviolet light in bone marrow-derived cells. *J Immunol* 2011;187:1664–73.
10. Barr KL, Konia TH, Fung MA. Lupus erythematosus-like imiquimod reaction: a diagnostic pitfall. *J Cutan Pathol* 2011;38:346–50.
11. Yokogawa M, Takaishi M, Nakajima K, *et al*. Epicutaneous application of toll-like receptor 7 agonists leads to systemic autoimmunity in wild-type mice: a new model of systemic Lupus erythematosus. *Arthritis Rheumatol Hoboken NJ* 2014;66:694–706.