A review of the mechanisms of action of dimethylfumarate in the treatment of psoriasis

Jürgen Brück1 | Ralf Dringen2,3 | Adriana Amasuno4 | Ignasi Pau-Charles4 | Kamran Ghoreschi1

Abstract
Fumaric acid esters (FAEs) such as dimethylfumarate (DMF) are used for the treatment of adults with moderate-to-severe psoriasis. The mode of action of FAEs is complex. Here, we provide a comprehensive review of the literature to describe the molecular mechanisms by which DMF and its active metabolite monomethylfumarate (MMF) exert their anti-inflammatory and immune modulatory effects. MMF can bind to the hydroxy-carboxylic acid receptor 2 (HCA2) on the cell surface and both DMF and MMF react with intracellular glutathione following cell penetration. DMF and to some extent also MMF modulate the activity of certain cellular signalling proteins such as the nuclear factor (erythroid-derived 2)-like 2 (Nrf2), nuclear factor kappa B (Nf-κB) and the cellular concentration of cyclic adenosine monophosphate. Some studies show that DMF can also affect the hypoxia-inducible factor 1-alpha (HIF-1α). These actions seem to be responsible for i) the downregulation of inflammatory cytokines and ii) an overall shift from a proinflammatory Th1/Th17 response to an anti-inflammatory/regulatory Th2 response. Both steps are necessary for the amelioration of psoriatic inflammation, although additional mechanisms have been proposed. There is a growing body of evidence to support the notion that DMF/MMF may also exert effects on granulocytes and non-immune cell lineages including keratinocytes and endothelial cells. A better understanding of the multiple molecular mechanisms involved in the cellular action of FAEs will help to adapt and further improve the use of such small molecules for the treatment of psoriasis and other chronic inflammatory diseases.

KEYWORDS
dimethylfumarate, fumaric acid esters, inflammation, monomethylfumarate, psoriasis

Abbreviations: AMPs, antimicrobial peptides; cAMP, cyclic adenosine monophosphate; CNS, central nervous system; DCs, dendritic cells; DMF, dimethylfumarate; FA, fumaric acid; FAEs, fumaric acid esters; GSH, glutathione; HaCaT, human keratinocyte cell line; hBD2, human β-defensin 2; HCA2, hydroxy-carboxylic acid receptor 2; HIF-1α, hypoxia-inducible factor 1-alpha; HO-1, heme oxygenase-1; ICAM, intercellular adhesion molecule; JAK, Janus kinase; MEF, monomethylfumarate; MMF, monomethylfumarate; MS, multiple sclerosis; Nrf2, nuclear factor (erythroid-derived 2)-like 2; PK, pharmacokinetic; PML, progressive multifocal leukoencephalopathy; ROS, reactive oxygen species; STAT, signal transducer and activator of transcription; Th cell, T-helper cell; TLR, Toll-like receptor; VCAM, vascular cell adhesion protein; VEGF, vascular endothelial growth factor.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2018 The Authors. Experimental Dermatology Published by John Wiley & Sons Ltd
1 | INTRODUCTION

1.1 | Psoriasis: aetiology, pathogenesis and treatment

Psoriasis is a chronic inflammatory disease of the skin.\(^1,2\) Psoriatic lesions can be itchy and painful, and may cause extreme physical and emotional discomfort and reduce patients’ quality of life.\(^2,5\) In the most severe cases, patients with psoriasis have an increased risk of developing serious comorbidities,\(^4,5\) which can eventually increase the overall risk of morbidity and mortality.\(^2,4\)

While the aetiology of psoriasis remains to be fully elucidated, it is considered an immune-mediated disorder. A combination of genetic, immunological and environmental factors contributes to the phenotype of psoriasis.\(^2,3,7,8\) Historically, psoriatic skin is infiltrated by T cells\(^2,5,9\) and neutrophils, which form Munro’s microabscesses in the epidermis.\(^10\) The pathogenesis of psoriasis is primarily mediated by T cells and dendritic cells (DCs); the aberrant induction of immune cells and subsequent excessive release of inflammatory cytokines and chemokines (such as interleukin [IL]-23, IL-17, and tumor necrosis factor [TNF]) from psoriatic skin promotes further recruitment of immune cells, hyperproliferation of keratinocytes, endothelial cells and sustained chronic inflammation.\(^7,9,11-12\) While IL-23 is mainly produced by infiltrating DCs,\(^14\) the Th17 cytokines in psoriatic lesions are produced by multiple immune cells such as CD4\(^+\) T-helper cells, CD8\(^+\) cytotoxic T cells, innate lymphoid cells and mast cells. IL-23 is a key regulator of IL-17-secretion.\(^15\) In vitro studies in murine CD4\(^+\) T cells have shown that IL-23 is able to maintain the Th17 phenotype of purified IL17\(^+\) cells; however, this is not sufficient to affect proliferation or survival of these cells.\(^16\) IL-17 and its associated cytokine IL-22 promote the production of IL-8 and granulocyte colony-stimulating factor, which recruit neutrophils to the site of inflammation and in turn promote the production of antimicrobial peptides (AMPs) by human keratinocytes. The AMP LL37 has been suggested to act as an autoantigen in psoriasis.\(^17\) LL37-reactive T cells produce interferon (IFN)-γ and IL-17, indicative of a Th17-type response.\(^17\)

Recently, it has been shown that the formation of cytosolic RNA:DNA complexes in keratinocytes within psoriatic lesions can also activate the production of inflammatory cytokines.\(^18\) The complexes formed between AMPs and free self-DNA or self-RNA can also act as autoantigens and activate DCs through Toll-like receptors (TLR) such as TLR7, TLR8 and TLR9.\(^19\)

Constant AMP overexpression in psoriatic skin perpetuates the psoriatic inflammation and plaque formation.\(^20\) Interestingly, the human leucocyte antigen (HLA)-C class I allele HLA-C*06:02, which is highly associated with the susceptibility for psoriasis, has been shown to present a melanocytic autoantigen (ADAMTS-like protein 5) to CD8\(^+\) lymphocytes.\(^21\) In terms of genetic predisposition, the psoriasis susceptibility locus PSORS1 (encoding HLA-C) was identified in 2006 as the main psoriasis risk allele.\(^22\) More recently, exome-wide genetic association studies have discovered a new susceptibility locus at TNFSF15.\(^23\) Besides these loci, polymorphisms in gene loci with a confirmed association with psoriasis include genes involved in IL-23 biology (IL23A, IL23R and IL12B), genes that act downstream of TNF and regulate nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signalling (TNIP1, TNFAIP3), genes involved in the modulation of Th2 immune responses (IL4, IL13)\(^24\) as well as genes involved in type 1 IFN signalling (IFIH1, TYK2).\(^23\) Subsequently identified loci have increased the number of psoriasis-associated genes to 36, including genes whose products regulate T-cell function, including Th17 responses (e.g. RUNX3, TAGAP and STAT3). Such gene products are also involved in innate immunity, encoding proteins with roles in IFN-mediated antiviral responses (DDX58), macrophage activation (ZC3H12C) and NF-κB-related signalling (CARD14 and CARM1).\(^25\) Single nucleotide polymorphisms in the TNF receptor-associated factor 3 interacting protein 2 (TRAF3IP2, which encodes Act1, the adaptor protein of IL-17 signalling) have been correlated with altered modulation of immunoregulatory signals through altered TRAF interactions, leading to increased susceptibility to psoriasis and psoriatic arthritis.\(^26\) Moreover, genes encoding signalling molecules downstream of the IL-23 receptor (TYK2, STAT3) are closely linked to the risk of developing psoriasis. In some patients with generalized pustular psoriasis, mutations within the IL-36 receptor antagonist gene (IL36RN) are found.\(^27\)

Available treatments for psoriasis are prescribed according to assessment of disease severity by clinical scores and quality-of-life questionnaires,\(^28,29\) among other factors; mild psoriasis is most commonly treated with topical agents, either as a monotherapy or in combination with phototherapy.\(^30\) Moderate-to-severe psoriasis is usually treated with systemic agents (e.g. methotrexate, cyclosporin, acitretin, apremilast, fumaric acid esters [FAEs]) and biologic treatments, which target specific inflammatory cytokines such as IL-12/IL-23p40, IL-23p19, IL-17A or TNF, respectively.\(^28,29\) FAEs have become a popular choice for the systemic treatment of psoriasis, particularly in Germany, but have also grown in popularity elsewhere in Europe.\(^31\)

1.2 | Fumaric acid esters

FAEs are lipophilic ester derivatives of fumaric acid (FA) that were first shown to exert therapeutic properties in psoriasis by the German biochemist Dr Schweckendiek over 50 years ago.\(^32\) These findings sparked several small-scale studies with empirical combinations of various types of orally administered FAEs. FA and FAE tablets given to patients with psoriasis significantly correlated with clearance of the skin within weeks.\(^33\) However, FA alone was later confirmed not to have any effect upon psoriasis.\(^34\) Instead, it was the esters of FA that provided antipsoriatic activity.\(^34,35\) Since then, FAEs have been used to treat psoriasis for many decades, primarily in Germany and some other European countries. A mixture of four FAEs (dimethylfumarate [DMF] and three salts of monoethylfumarate [MEF, also known as ethylhydrogenfumarate])
eventually gained approval in Germany as an oral treatment (Fumaderm®) for moderate-to-severe psoriasis vulgaris. More recently, formulations containing DMF as monocompound (BG12, originally owned by Biogen Idec, and LAS41008, Almirall S.A.) have been tested in the setting of psoriasis. Recently, the first gastro-resistant oral formulation of DMF (Skilarence®) as monocompound was granted marketing authorisation for the treatment of adults with moderate or severe chronic plaque psoriasis by the European Medicines Agency. A summary of the dosing, administration, laboratory monitoring and possible side effects of Skilarence® and Fumaderm® is given in Table 1.

In the latest European psoriasis treatment guidelines, FAEs are strongly recommended as systemic therapy for both the induction and the long-term treatment of patients with moderate-to-severe chronic plaque psoriasis. Due to its recent approval, the use of DMF as a monotherapy for moderate-to-severe psoriasis is yet to be discussed specifically in European guidelines; however, treatment guidelines will likely continue to evolve as experience with these agents grows.

### TABLE 1 DMF (Skilarence®) and FAEs (Fumaderm®) details of dose, treatment administration, laboratory monitoring and possible side effects (according to the respective SmPC)

| Skilarence SmPC | Fumaderm SmPC |
|-----------------|----------------|
| **Composition** | Skilarence 30 mg (30 mg DMF)  
Skilarence 120 mg (120 mg DMF) | Fumaderm Initial 30 mg  
(30 mg DMF, other FAE salts also [MEF])  
Fumaderm (120 mg DMF, other FAE salts also [MEF]) |
| **Pharmaceutical form** | Gastro-resistant tablet | Enteric-coated tablet |
| **Posology and method of administration** | Week 1: Skilarence 30 mg q.d.  
Week 2: Skilarence 30 mg b.i.d.  
Week 3: Skilarence 30 mg t.i.d.  
Week 4: Switch to Skilarence 120 mg This dose is then increased by one Skilarence 120 mg tablet per week to a maximum daily dose of 720 mg DMF (3 × 2 tablets of Skilarence 120 mg). | Week 1: Fumaderm Initial q.d.  
Week 2: Fumaderm Initial b.i.d.  
Week 3: Fumaderm Initial t.i.d.  
Week 4: Switch to Fumaderm once daily  
Depending on tolerance, this is increased by one Fumaderm tablet per week to a maximum daily dose of 720 mg DMF (3 × 2 tablets of Fumaderm). |
| **Laboratory monitoring** | Prior to treatment  
• Complete blood count  
• Treatment should not be initiated if leucocyte count is <3.0 × 10^9/L, lymphocyte count <1.0 × 10^9/L, or other pathological results are identified  
During treatment  
• Complete blood count every 3 mo.  
• If lymphocyte counts fall <1.0 × 10^9/L but are ≥0.7 × 10^9/L, blood monitoring should be performed monthly until levels return to ≥1.0 × 10^9/L for two consecutive tests.  
• If lymphocyte counts fall below 700/μL a retest has to be performed and if lymphocytes are still <0.7 × 10^9/L treatment has to be stopped.  
• Treatment should be stopped if leucocyte levels fall <3.0 × 10^9/L. | Prior to treatment  
• Complete blood count  
• Treatment should not be initiated if blood counts outside of the normal range  
During treatment  
• Complete blood count every 4 wk.  
• If lymphocyte counts fall <500/μL, treatment must be stopped.  
• If lymphocyte counts fall <700/μL, the dose must be halved and stopped after 4 wk if no improvement is observed.  
• Treatment should be stopped if leucocyte levels fall <3.0 × 10^9/L. |
| **Possible side effects** | “Very common” adverse events include gastrointestinal disorders (diarrhoea, abdominal pain and nausea), flushing, leukaemia and lymphopenia. | “Very common” adverse events include gastrointestinal disorders (diarrhoea), flushing, mild leukaemia and mild lymphopenia. |

*Monitoring hepatic function (liver enzymes) and renal function (creatinine, urinalysis) is also recommended prior to and during treatment.

2 | REVIEW OBJECTIVES

FAEs are thought to mediate their antipsoriatic effects by activating various cellular pathways that either lead to the induction of T-cell apoptosis or that prevent the release of proinflammatory cytokines, but their mode of action has not yet been completely elucidated. DMF and MEF are pharmaceutically different (Table 2), with distinct properties in terms of chemical structure and molecular weight, as well as melting points, water solubility and acid dissociation constants. DMF is thought to be the main active FAE in the treatment of psoriasis, according to both preclinical and clinical evidence.

This comprehensive literature review explores the pharmacokinetics (PK) and mode of
action of DMF, followed by an analysis of its biological actions in the context of psoriasis.

3 | PHARMACOKINETICS OF DMF

To date, it remains unclear to what extent DMF or its main metabolite monomethylfumarate (MMF) are active in psoriasis, as most studies have not systematically evaluated all the different FAE compounds in direct comparison with each other. Early investigations suggested that DMF was a prodrug for MMF,[48] however, subsequent studies have shown that DMF and MMF may not be totally therapeutically equivalent: DMF has displayed distinct pharmacological properties in certain experimental models (eg effects on downstream molecular signalling and gene activation properties), compared with MMF,[45,49] and, therefore, some authors consider that DMF is not just a prodrug of MMF.[45,49] However, the in-vivo relevance of these findings has yet to be elucidated. In addition, it has been shown that DMF is rapidly hydrolysed to MMF in vivo and in vitro and that there is no re-esterification of MMF to DMF.[50,51]

Upon ingestion and exposure to isolated gastrointestinal mucosa, most of the ingested DMF is rapidly converted to MMF (Figure 1).[52] This rapid hydrolysis of DMF to MMF partly accounts for the lack of detectable levels of DMF in the circulation, as shown in phase I PK studies. In healthy subjects receiving DMF plus calcium MEF (120 mg plus 95 mg) under fasting conditions, MMF transiently increased to a median maximal plasma concentration of 0.84 mg/L and then disappeared from the plasma with a median elimination half-life of 44 minutes, whereas DMF and FA could not be detected.[53]

Similarly, in three psoriasis patients who were being treated with the approved FAE mixture, peak concentrations of MMF were observed after 3.5-5.0 hours and declined with a half-life of 31-71 minutes, while DMF plasma levels were undetectable,[50] which could be partly due to its lipophilic properties. Several studies to date have shown that MMF levels in the plasma rise sharply and decline rapidly. Indeed, Dibbert et al reported rapid DMF metabolism in vivo.[51]

After oral administration, DMF and MEF are hydrolysed by esterases in the gut. The fumarate generated, as well as the acetate formed by hydrolysis of MEF, is subsequently metabolized via the citric acid cycle,[53,54] while methanol derived from DMF and MMF is oxidized via formaldehyde and formate to carbon dioxide (CO₂). Thus, the main excretion route of metabolized FAEs appears to be CO₂ exhalation. Whether metabolic products such as methanol or methanol-derived formaldehyde which affect glutathione (GSH) metabolism and neuronal diseases also influence psoriasis pathology is unclear.[55]
Analyses of urine samples from psoriasis patients treated with the approved FAE mixture suggest that DMF is not completely hydrolysed upon ingestion, but that a small proportion is absorbed into the presystemic circulation. The involvement of circulating cells in the hydrolysis of DMF to MMF is supported by the shorter in vitro half-life of DMF in whole blood (0.10 hours) compared with plasma (0.37 hours).

Both DMF and MMF are known to interact with free thiols or cysteine residues at physiological pH, and because of this, DMF, and to a smaller extent MMF, reacts with intracellular and extracellular GSH. Absorbed DMF rapidly penetrates into blood cells including immune cells, where it covalently binds to GSH and/or other molecules. This spontaneous formation of DMF-GSH adducts is the other reason for not detecting DMF in the circulation.

By modulating the glutathione content, and therefore the redox state of cells, DMF/MMF influence intracellular signalling pathways. Mrowietz et al proposed a metabolic pathway which leads to GSH adducts of DMF and MMF (Figure 1). These adducts are further metabolized and finally excreted as acetylcysteine conjugates via the kidney. The fraction of DMF excreted via the kidneys, however, is a small percentage, compared with that excreted via exhalation.

Whilst the metabolism of DMF is well understood, there remains some debate over the respective biological activities of DMF and its metabolite MMF in vivo. Some studies have suggested that the effects of FAEs may not be attributed to DMF directly, as DMF is not detected in the circulation. DMF presumably interacts with the gut tissue and gut-associated cells. Its metabolite MMF but also the aforementioned GSH adducts of DMF and MMF may interact with organs and cells beyond the gastrointestinal system. There is, however, some evidence to indicate that DMF is able to elicit some direct effects in vivo and this may be supported by the finding that not all DMF is hydrolysed upon ingestion. In subsequent sections therefore, the activities of DMF and MMF have been distinguished separately.

4 | MECHANISM OF ACTION OF DMF

While DMF has been the primary fumarate used in most mechanism of action studies, it has been assumed, due to its short half-life in plasma, that its primary metabolite MMF mediates all the mechanistic effects of DMF. However, the relationship between the mechanisms of action of DMF and MMF is more complex. DMF (but not MMF) protects embryonic primary cortical cultures from C57BL/6 and SJL mice from oxidative glutamate toxicity and DMF demonstrates a more potent cytoprotective effect than MMF in neurons and astrocytes in vitro, via activation of the nuclear factor (erythroid-derived 2)-like 2 (Nrf2) pathway. Other preclinical and clinical studies have also indicated a higher biological potency for DMF compared with MEF, which is another type of FAE. These findings suggest that DMF and MMF could have some separate actions in vivo, although further research is needed.

Currently, there are at least five main mechanisms for the general action for DMF/MMF that have been described so far, with close interaction with each other. These mechanisms are summarised in Table 3.

1. After cellular uptake, the α, β unsaturated carboxylic acid ester DMF reacts with thiol groups of GSH and thereby lowers GSH levels. This impacts cellular responses to oxidative stress.

2. Activation of the Nrf2-dependent antioxidant response pathway leads to stimulation of cytoprotective and anti-inflammatory genes.

3. Direct and/or indirect inhibition of NF-κB activity affects cytokine production, the phenotype of antigen-presenting cells and subsequently shifts the Th1/Th17 immune response to a Th2 phenotype.

4. MMF is an agonist for the G protein-coupled receptor 109A (GPR109A, also known as the hydroxy-carboxylic acid receptor 2 [HCA2]) that influences neutrophil adhesion, migration and recruitment. This action is also believed to be related to the flushing side effect that is commonly reported in patients taking DMF.

5. Modulation of oxidative stress-sensitive transcription factors such as hypoxia-inducible factor 1-alpha (HIF-1α) and signal transducers and activators of transcription (STATs).

4.1 | Modulation of intracellular glutathione levels

In vivo, modulation of intracellular GSH levels would only occur in the small intestine mucosal cells upon ingestion of DMF. It is considered that some of the anti-inflammatory and immunomodulatory effects of DMF are mediated through its interaction with intracellular GSH (Figure 2). In vitro, DMF functionally depletes intracellular GSH in multiple cell types. This leads to an irreversible inhibition of Keap-1, the activation of Nrf2 and the induction of heme oxygenase 1 (HO-1), causing the downregulation of several inflammatory cytokines. The depletion of intracellular GSH in antigen-presenting cells such as human or mouse DC by DMF generates type II DCs that produce IL-10 instead of IL-12 and IL-23. This results in the inhibition of Th1/Th17 cells and promotes instead Th2 cell differentiation.

In turn, replenishing intracellular GSH levels by application of GSH ethyl ester or addition of GSH-precursors such as N-acetyl-L-cysteine diminishes and neutralizes the effect of DMF on DC cytokine production.

In summary, DMF can reduce the inflammation associated with psoriasis through the depletion of cellular GSH and thus driving an immune response from one of inflammation (Th1/Th17 response) to one that is anti-inflammatory (Th2) in the setting of psoriasis. In addition, GSH depletion by high concentrations of DMF induces cell death in different human cell types, including activated T cells, erythrocytes, colon cancer cells and cutaneous T-cell lymphoma (CTCL) cells.
4.2 | Activation of Nrf2

The transcription factor Nrf2 is normally retained in the cytoplasm through its interaction with Keap-1. The binding of DMF and MMF to cysteine residues of Keap-1 induces a conformational change that allows Nrf2 to dissociate from Keap-1 and enter the nucleus. [67] After DMF-induced translocation, Nrf2 regulates genes that prevent oxidative damage in cells of the nervous system [62] or in keratinocytes. [91] Specifically, the antioxidative and anti-inflammatory stress protein HO-1 (encoded by the gene Hmox1), a known target gene of Nrf2, is induced by DMF (Figure 2). Additionally, HO-1 is reported to stabilize Nrf2 to prolong its accumulation within the nucleus, thereby enhancing the antioxidative defense. [92,93]

The immunomodulatory effects of DMF in experimental settings of autoimmune inflammatory disorders such as multiple sclerosis (MS) are thought to be mediated through both Nrf2-dependent and Nrf2-independent pathways. [40,45,94,95] Pharmacological activation of Nrf2 may be a major strategy against MS, [96,97] other inflammatory diseases and against cancer. [98-100] Nrf2 is also important to protect keratinocytes from oxidative damage and DMF is a potent inducer of HO-1 in human keratinocytes cultured in vitro. [101,102] Although DMF is not detectable in the skin after oral application, GSH- and Nrf2-regulated genes are affected at the level of expression in psoriatic plaques of patients treated with DMF. [103] Some of these effects may be mediated by metabolites of DMF such as MMF. Recently, MMF has been reported to induce Nrf2 levels and like DMF, MMF promotes the expression of Nrf2-regulated genes like HO-1 in keratinocytes in vitro. [104]

Evidence of the antioxidant activity of DMF has also been observed in the central nervous system (CNS). Nrf2 activation is thought to be the mechanism by which DMF exerts its neuroprotective effects in MS. [67,105] For instance, DMF increases
Nrf2-dependent NAD(P)H: quinone reductase expression in neuronal cells in vitro and in vivo. FAEs have been shown to activate the Nrf2 antioxidant pathway in the CNS and other tissues such as cardiac cells. Treatment of CNS cells with DMF or MMF affects GSH levels, ATP levels, cellular redox potential, mitochondrial membrane potential and HO-1 expression in a concentration-dependent manner.

4.3 Inhibition of NF-κB activity

The NF-κB family consists of several different proteins, which all share a conserved Rel homology domain responsible for dimerization, nuclear localization and DNA binding. DMF has been suggested to inhibit the translocation of NF-κB family members into the nucleus in certain cell types such as normal human dermal fibroblasts, as well as in normal human keratinocytes, human endothelial cells and isolated peripheral blood mononuclear cells in vitro. This effect seems to be independent of Nrf2. Interestingly, equivalent doses of MMF and MEF did not affect NF-κB translocation and activity by direct or indirect mechanisms is discussed controversially. One indirect mechanism proposed is the inhibition of NF-κB p65 activity by DMF-induced nuclear HO-1 (Figure 2). Whether DMF affects NF-κB translocation and activity by direct or indirect mechanisms is discussed controversially. One indirect mechanism proposed is the inhibition of NF-κB p65 activity by DMF-induced nuclear HO-1 (Figure 2). However, DMF could suppress NF-κB target genes such as inflammatory cytokines (e.g., IL-6), chemokines (e.g., CCL17) and adhesion molecules (e.g., CD44 and neuronal cell adhesion molecule). Whether DMF affects intracellular GSH levels or on Nrf2 and NF-κB signalling cannot be explained by a receptor-mediated mechanism such as HCA2 binding (Figure 2).

4.4 HCA2 activation

HCA2 (also known as GPR109A or niacin receptor 1) is a high-affinity G protein-coupled receptor that is primarily expressed in adipocytes, and in other cells types such as keratinocytes, neutrophils, macrophages and Langerhans cells. GPR109A is thought to be implicated in certain inflammatory pathways. Of note, HCA2 expression is upregulated in psoriatic skin. MMF has been reported to be a HCA2 agonist leading to reduced neutrophil adhesion, migration and recruitment (Figure 3). One recent study suggested that the protective effects of DMF in a mouse model of MS are mediated through HCA2. HCA2 agonism, which induces PGE2 production in keratinocytes, may also be responsible for the flushing symptoms that are observed in patients undergoing FAE therapy. However, the effects of DMF on intracellular GSH levels or on Nrf2 and NF-κB signalling cannot be explained by a receptor-mediated mechanism such as HCA2 binding (Figure 2).
expression of proangiogenic genes.\textsuperscript{[13,118]} DMF has been reported to impair the hypoxia-induced protein expression of HIF-1α by promoting its degradation (Figure 2). As a consequence, the expression of HIF-1α target genes such as IL-8 and vascular endothelial growth factor (VEGF) is affected by DMF.\textsuperscript{[84]}

Some of the actions of DMF affect the Janus kinase (JAK)/signal transducers and activators of transcription (STAT) signalling pathway (Figure 2).\textsuperscript{[85,86]} In DCs, DMF treatment stimulates apoptosis\textsuperscript{[119]} and DMF has also been shown to inhibit the activation of STAT1 and to impair IL-12 production; its effect on STAT1 depends on the DCs’ intracellular GSH level.\textsuperscript{[27]} In addition, Nrf2-dependent induction of HO-1 enhances STAT3 activation in DC, a negative regulator of Il23a transcription.\textsuperscript{[97]} Additional studies to examine the antioxidant properties of DMF and its consequences on signalling pathways in more depth are desirable.

Other signalling factors proposed to be affected by DMF in immune cells include indolamine 2,3-dioxygenase (IDO),\textsuperscript{[120]} TLR-induced M1 and K63 ubiquitin chain formation\textsuperscript{[121]} and the stimulation of cAMP signalling.\textsuperscript{[122]}

### 4.6 T-helper cell responses in psoriasis and their modulation

Psoriatic lesions are characterized by a strong presence of Th1/Th17 cells\textsuperscript{[123]} and a relative absence of Th2 cells.\textsuperscript{[124,125]} The Th2 lineage-defining cytokine IL-4 is a key factor, essential for Th2 differentiation\textsuperscript{[126]} and has anti-inflammatory effects by down-regulating IL-1, TNF, IL-6, IL-8, IL-12, IL-17 and IL-23 in different cell types.\textsuperscript{[127–129]} The epidermal alterations in psoriatic lesional skin include increased epidermal expression of IL-1,\textsuperscript{[130]} IL-6,\textsuperscript{[131]} and AMPs such as psoriasin (S100A7) and human β-defensin 2 (hBD2),\textsuperscript{[2]} as well as a downregulated expression of the epidermal transcription factor GATA3.\textsuperscript{[132]} Onderdijk et al showed that IL-4 directly inhibits these psoriatic markers in the epidermal compartment.\textsuperscript{[133]} IL-4 reduced the expression of IL-1, IL-6 and hBD2, while phospho-STAT6 and GATA3 were significantly upregulated.\textsuperscript{[133]} These findings suggest that IL-4 improves psoriasis not only through induction of type II dendritic cells and Th2 cells, but also through direct inhibition of inflammatory cytokines in resident IL-4R-expressing epidermal cells, thereby shifting the psoriatic skin phenotype towards a “healthy” skin phenotype.\textsuperscript{[133]} Likewise, DMF and MMF formulations also induce Th2 markers such as GATA3 in the skin of patients with psoriasis.\textsuperscript{[103]}

Moreover, IL-4 is a negative regulator of Th17 cell differentiation. On the one hand, IL-17A transcription by CD4⁺ Th cells is inhibited by IL-4/STAT6 signalling.\textsuperscript{[124]} On the other hand, IL-4 affects cytokine production by DC. While IL-4 promotes IL-12–production by DCs, it abrogates IL-23 expression in this cell type. This selective silencing of IL-23/Th17 responses by IL-4 could be a physiologically relevant target.\textsuperscript{[135]} In fact, IL-4 treatment of humans with plaque-type psoriasis improves the disease significantly and induces Th2 cells.\textsuperscript{[125,136]} Other clinical studies have also indicated a role for Th2-associated cytokines such as IL-10\textsuperscript{[137–141]} and IL-11\textsuperscript{[142]} in the treatment of psoriasis. DMF also induces IL-4⁺ Th2 cells as shown in mouse models of MS and in patients treated for psoriasis, as demonstrated in a placebo-controlled randomized study.\textsuperscript{[97]}

### 5 Biological effects on cell types relevant to psoriasis

Many of the data describing the biological actions of DMF have been obtained from in vitro studies. Given the complex PK profile of this compound and the fact that many cell types and molecular pathways are involved in the pathogenesis of psoriasis, the relevance of these biological effects in an in vivo situation is less clear. Notably, it must be considered that the majority of the ingested DMF may not actually get to the skin, and that the small amount of DMF that enters the circulation may exert its actions by modulating GSH metabolism and various intracellular signalling pathways in peripheral tissue or circulating immune cells. In addition, MMF may contribute to the beneficial effects of its prodrug DMF by binding to the HCA2 receptor. Taken together, the effects of DMF may be essentially immunologically based, at least as reflected by recent studies in psoriasis and MS.\textsuperscript{[143,144]} Effects that have been described on other cell types (such as endothelial cells and keratinocytes) may in fact be of little relevance to the clinical effect of DMF in psoriasis, when taken orally. Yet, we summarize in the following the biological effects of DMF on different cell types that are important for the pathogenesis of psoriasis.

#### 5.1 Lymphocytes

Resident T lymphocytes, macrophages and neutrophils infiltrate psoriatic lesions before the development of significant epidermal changes.\textsuperscript{[145,146]} The involvement of T lymphocytes in psoriasis occurs in three stages: initial activation, migration into the skin and the release of inflammatory cytokines.\textsuperscript{[7]} FAEs are thought to mediate their antipsoriatic effects by activating cellular pathways that lead to the induction of apoptosis in immune cells or that prevent the release of inflammatory cytokines (Figure 3). In vitro, DMF is a potent apoptotic agent when used in high concentrations.\textsuperscript{[42]} DMF also impairs T-cell cytokine secretion.\textsuperscript{[147]} Both, the apoptosis-inducing properties and the impairment of cytokine secretion seem to be mediated through NF-κB inhibition.\textsuperscript{[142,75,148,149]}

In patients treated with FAEs, a decrease in total leucocyte counts (especially lymphocytes) can occur in peripheral blood. Höxtermann and colleagues reported a reduction (48.7%) in lymphocyte counts in a small cohort of ten patients during the first 3 months of treatment with FAEs following a fixed regimen with increasing dosages.\textsuperscript{[150]} A more recent single-centre observational study documented the lymphocyte counts of 105 patients treated with FAE. They found a reduction in lymphocyte counts in 30.5% of patients after 6 months of treatment and a severe reduction (<500/μL) in 2.9% of patients within the first 6 months.\textsuperscript{[151]} In MS patients treated with DMF, a selective reduction in memory T cells has been
reported. However, the in vivo mechanism remains unclear. While DMF inhibits NF-κB in activated human T cells, no effects were observed on nuclear factor of activated T cells or cytosine-adenine-guanine dinucleotide box motif/enhancer-binding protein beta. Other studies have further demonstrated that DMF (but not MMF) inhibits allantocrine T-cell proliferation and induces apoptosis in human T cells in vitro. Additional effects of DMF on leukocytes involve reduced leucocyte rolling in vivo, through modulation of adhesion molecule expression, inhibition of integrin α4 expression through the inhibition of inflammatory cytokines, and the induction of IL-4 producing T cells in humans with psoriasis or MS. In turn, the modulation of inflammatory cytokines by DMF leads to a shift from Th1/Th17 towards Th2 cell populations, resulting in an anti-inflammatory response. An immunohistochemical study in six patients with psoriasis who received DMF monotherapy (720 mg/d) for 16 weeks showed that DMF significantly reduced lesional T-cell subsets, normalized epidermal hyperproliferation, keratinization, and reduced epidermal thickness.

Clinical trials of DMF therapy for the MS indication have reported a 4%-5% occurrence of grade 3 lymphopenia (lymphocyte counts <0.5 x 10^9 cells/L). Additional postmarketing studies with DMF in MS have also reported up to 50% occurrence of lymphopenia, with a more prominent reduction in CD8+ vs CD4+ T-cell counts. Studies in the setting of MS have suggested that T-cell subsets may have different susceptibilities to apoptosis induced by DMF. Of note, some cases of progressive multifocal leukoencephalopathy (PML) have been described in patients taking FAEs. In most of these cases, persistent lymphopenia was also reported. Important to note that in all reported cases of lymphopenia, the recommended rules for monitoring blood counts in patients (as described in current guidelines) were not sufficiently adhered to and this resulted in long periods of unknown lymphocyte counts. The European Medicines agency has recently reviewed cases of PML in patients receiving Fumaderm® and Psorinovo®, two FAE-based formulations used to treat psoriasis. Taken together, these data strengthen the need for regular monitoring of patients receiving DMF. The EMA has since made recommendations for the regular blood cell count analysis in patients receiving DMF, including treatment discontinuation in severe cases.

5.2 Neutrophils

As neutrophils also constitute a significant portion of infiltrating cells in psoriasis, some studies have examined the in-vitro and in-vivo effects of DMF on granulocytes such as neutrophils (Figure 3). In vitro, DMF inhibited neutrophil activation by inducing changes in surface marker expression and reactive oxygen species production and by impairing migration and phagocytic ability. This action of DMF on neutrophils seems to be mediated via different pathways, including the PI3K/Akt-p38 MAPK- and ERK 1/2 pathways, most prominently via cytokine-induced phosphorylation of these molecules, and this may represent an alternative mode of action of DMF, independent of those already reported. Additional work by Chen and colleagues in a mouse model of MS has confirmed that DMF is able to decrease the number of infiltrating neutrophils in a HCA2-dependent manner and is most likely a result of aberrant neutrophil adhesion to endothelial cells and chemotaxis. More recently, DMF was also reported to inhibit extracellular trap formation by neutrophils. This effect was dependent on reactive oxygen species (ROS) and GST.
formation in VEGF-stimulated human endothelial cells in vitro. This effect is associated with a reduced expression of VEGF receptor 2 by DMF treatment[171]. Following reports that angiogenesis is a key driver of psoriasis pathogenesis, Garcia-Caballero and colleagues have reported that DMF is able to inhibit angiogenesis (tubule formation) both in vitro and in vivo in the quail chorioallantoic membrane and a transgenic zebrafish, respectively. This may partially explain its role as an antipsoriatic agent. Interestingly, neither MMF nor free FA displayed antiangiogenic activity in this context.[172,173] Inhibition of angiogenesis might play a role in the activity of DMF, given that persistent angiogenesis is associated with several skin disorders. However, other studies have found no effect of DMF or MMF on the basal IL-1β-inducible expression of ICAM-1 on brain endothelial cells.[110]

6 | SUMMARY

FAEs are a valuable oral systemic treatment for patients with moderate-to-severe chronic plaque psoriasis. Accumulating evidence from preclinical studies,[43,44,69,74,149,164] as well as pharmacological[45] and clinical studies with DMF and other FAEs[46,174,175], emphasizes that DMF is the main antipsoriatic compound among the different FAEs tested.

Whilst the anti-inflammatory and immunomodulatory effects of DMF are not completely understood, there are at least five main general mechanisms that have been put forward to explain its antipsoriatic action. DMF and its active metabolite MMF may act via several pathways and seem to involve both immunomodulatory and antioxidative actions, including activation of Nrf2 to stimulate anti-inflammatory pathways,[66–68] inhibition of NF-κB-driven processes,[37,69–77] modulation of GSH levels and ultimately cellular response to oxidative stress[43,48,51,56,58–61] and agonism of HCA2.[80–83] These mechanisms offer a means by which DMF can exert its antipsoriatic activity, and allow DMF to modulate the antioxidant defenses of cells and the inflammatory pathways that may exacerbate psoriasis. DMF can suppress the activity of NF-κB target genes such as inflammatory cytokines, chemokines and adhesion molecules that would otherwise drive a psoriatic phenotype. Depletion of cellular GSH levels in combination with this will further drive an immune response to an anti-inflammatory one, such that proinflammatory responses such as Th1/Th17 that are responsible for psoriasis are shifted to a non-pathogenic Th2 response in the setting of psoriasis.[37,77–79]

By the nature of its action within the body, the in vivo activity of DMF is thought to modulate the immune response.[143,144] In addition, the effects of DMF/MMF on non-immune cell lineages including keratinocytes may also be of relevance, as psoriasis is characterized by an epidermal hyperproliferation. Whilst there is in vitro evidence to demonstrate the effects of DMF on non-immune cell lineages, the complex PK profile of DMF makes it challenging to fully elucidate its effects in vivo. Despite this, the large amount of in vitro data collected offers much insight into the biological effects of DMF and the ways it executes its antipsoriatic effects. The main molecules that are influenced by DMF and that seem to be responsible for transmitting its actions within the cell are the classical signalling molecules Nrf2 and NF-κB, as well as intracellular GSH levels and cytoprotective proteins such as HO-1. Further research is eagerly awaited on the other recently described signalling cascades that are also modulated by DMF and MMF, involving molecules such as HCA2, cAMP, JAK/STAT, IDO and HIF-1α.

ACKNOWLEDGEMENTS

KG is supported by the Deutsche Forschungsgemeinschaft (DFG) Sonderforschungsbereich (SFB) TR-156, TP A06. Medical writing assistance was provided by Sandra Cuscó PhD of Bioscript Group, Macclesfield, UK and funded by Almirall S.A.

CONFLICT OF INTEREST

KG has been a consultant, lecturer or investigator for AbbVie, Almirall, Boehringer, Biogen, Celgene, Eli Lilly and Company, Janssen-Cilag, MSD Sharp & Dohme, Novartis Pharmaceuticals, and Pfizer. IP-C and AA are employees of Almirall S.A., Barcelona, Spain.

AUTHOR CONTRIBUTIONS

JB, RD, AA, IP-C and KG drafted and edited the paper jointly, reviewed it critically and approved the final submitted version.

ORCID

Kamran Ghoreschi http://orcid.org/0000-0002-5526-7517

REFERENCES

[1] C. E. Griffiths, E. Christophers, J. N. Barker, R. J. Chalmers, S. Chimenti, G. G. Krueger, C. Leonard, A. Menter, J. P. Ortonne, L. Fry, Br. J. Dermatol. 2007, 156, 258.
[2] F. O. Nestle, D. H. Kaplan, J. Barker, N. Engl. J. Med. 2009, 361, 496.
[3] A. Menter, C. E. Griffiths, Lancet 2007, 370, 272.
[4] S. N. Cohen, S. E. Baron, C. B. Archer, British Association of Dermatologists; Royal College of General Practitioners, Clin. Exp. Dermatol. 2012, 37(Suppl 1), 13.
[5] H. T. Chong, Z. Kopecki, A. J. Cowin, Biomed. Res. Int. 2013, 2013, 168321.
[6] E. Salahadeen, C. Torp-Pedersen, G. Gislason, P. R. Hansen, O. Ahlehoff, J. Eur. Acad. Dermatol. Venereol. 2015, 29, 1002.
[7] G. Krueger, C. N. Ellis, J. Am. Acad. Dermatol. 2005, 53, S94.
[8] U. Mrowietz, P. Altmeyer, M. Augustin, W. H. Boehncke, B. Bonnekoh, Y. Frambach, T. Gambichler, K. Ghoreschi, M. Hertl, A. C. Hund, A. Jacobi, A. Kuhn, R. J. Ludwig, T. Luger, S. F. Martin, H. Merk, J. Norgauer, K. Reich, M. Rostami-Yazdi, R. Sabat, K. Schäkel, K. Scharrfetter-Kochanek, M. P. Schön, N. Scola, M. Sticherling, D. Thaci, D. Wilsman-Theis, A. Viehweg, G. Wozel, C. C. Zouboulis, M. Neureither, J. Dtsch. Dermatol. Ges. 2012, 10(Suppl 8), 1.
[155] S. Zoghi, Z. Amirghofran, A. Nikseresht, N. Ashjazadeh, E. Kamali-Sarvestani, N. Rezaei, *Immunol. Invest.* 2011, 40, 581.

[156] S. Tahvili, B. Zandieh, Z. Amirghofran, *Int. J. Dermatol.* 2015, 54, e254.

[157] C. C. Gross, A. Schulte-Mecklenbeck, S. Klingsing, A. Posevitz-Fejfár, H. Wiendl, L. Klotz, *Neur. Neuroimmunol. Neuroinflamm.* 2016, 3, e183.

[158] H. J. Bovenschen, A. M. Langewouters, P. C. van de Kerkhof, *Am. J. Clin. Dermatol.* 2010, 11, 343.

[159] R. Gold, L. Kappos, D. L. Arnold, A. Bar-Or, G. Giovannoni, K. Selmaj, C. Tornatore, M. T. Sweetser, M. Yang, S. I. Sheikh, K. T. Dawson, *DEFINE Study Investigators,* *N. Engl. J. Med.* 2012, 367, 1098.

[160] R. J. Fox, D. H. Miller, J. T. Phillips, M. Hutchinson, E. Havrdova, M. Kita, M. Yang, K. Raghupathi, M. Novas, M. T. Sweetser, V. Viglietta, K. T. Dawson, *CONFIRM Study Investigators,* *N. Engl. J. Med.* 2012, 367, 1087.

[161] C. M. Spencer, E. C. Crabtree-Hartman, K. Lehmann-Horn, B. A. Cree, S. S. Zamvil, *Neur. Neuroimmunol. Neuroinflamm.* 2015, 2, e76.

[162] M. Ghadiri, A. Rezk, R. Li, A. Evans, F. Luessi, F. Zipp, P. S. Giacomini, J. Antel, A. Bar-Or, *Neur. Neuroimmunol. Neuroinflamm.* 2017, 4, e340.

[163] D. M. W. Balak, E. Hajdarbegovic, W. M. Bramer, H. A. Neumann, H. B. Thio, *J. Eur. Acad. Dermatol. Venereol.* 2017, 31, 1475.

[164] J. H. O. Hoffmann, K. Schaekel, D. Hartl, A. H. Enk, E. N. Hadaschik, *Br. J. Dermatol.* 2018, 178, 207.

[165] P. H. Nibbering, B. Thio, T. P. Zomerdijk, A. C. Bezemer, R. L. Beijersbergen, R. van Furth, *J. Invest. Dermatol.* 1993, 101, 37.

[166] J. G. Van der Schroeff, C. Oudshoorn, W. M. Nugteren-Huying, M. Ponec, *J. Invest. Dermatol.* 1989, 92, 537A.

[167] I. Helwa, R. Patel, P. Karemellas, I. Kaddour-Djebbar, V. Choudhary, W. B. Bollag, *J. Pharmacol. Exp. Ther.* 2015, 352, 90.

[168] B. Sebük, B. Bonnekoeh, R. Vetter, I. Schneider, H. Gollnick, G. Mahrle, *Eur. J. Dermatol.* 1998, 8, 29.

[169] B. Bonnekoeh, R. Bockelmann, A. Ambach, H. Gollnick, *Skin Pharmacol. Appl. Skin Physiol.* 2001, 14, 217.

[170] R. Heidenreich, M. Röcken, K. Ghoreschi, *Drug News. Perspect.* 2008, 21, 97.

[171] M. Meissner, M. Doll, I. Hrgovic, G. Reichenbach, V. König, T. Hailemariam-Jahn, J. Gille, R. Kaufmann, *J. Invest. Dermatol.* 2011, 131, 1356.

[172] M. Garcia-Caballero, M. Mari-Beffa, M. A. Medina, A. R. Quesada, *J. Invest. Dermatol.* 2011, 131, 1347.

[173] J. L. Arbiser, *J. Invest. Dermatol.* 2011, 131, 1189.

[174] C. Nieboer, D. de Hoop, P. N. Langendijk, A. C. van Loenen, J. Gubbels, *Dermatologica 1990*, 181, 33.

[175] M. W. M. W. Balak, E. Hajdarbegovic, W. M. Bramer, H. A. Neumann, H. B. Thio, *J. Eur. Acad. Dermatol. Venereol.* 2017, 31, 1475.

[176] M. Meissner, E. M. Valesky, S. Kippenberger, R. Kaufmann, *J. Dtsch. Dermatol. Ges.* 2012, 10, 793.

How to cite this article: Brück J, Dringen R, Amasuno A, Pau-Charles I, Ghoreschi K. A review of the mechanisms of action of dimethylfumarate in the treatment of psoriasis. *Exp Dermatol.* 2018;27:611–624. https://doi.org/10.1111/exd.13548