1521-0103/384/2/245–247$35.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS

dx.doi.org/10.1124/jpet.122.001469
J Pharmacol Exp Ther 384:245–247, February 2023

Viewpoint

In Vitro Trials: The Dawn of a New Era for Drug Discovery in Atopic Dermatitis?

Atopic dermatitis (AD) is a chronic, pruritic inflammatory skin disease, affecting up to 25% of children and about 10% of adults, including 1-3% of the elderly. It presents with variable clinical phenotypes and is characterized by eczematous papules and plaques with excoriation, exudation and/or lichenification, intense itching and xerosis (Ständer, 2021). Over the past decade, increasing understanding of AD pathogenesis has led to the discovery of new therapeutic targets (Chovatiya and Paller, 2021; Ständer, 2021). Monoclonal antibodies that inhibit the IL-4 and/or IL-13 pathway have revolutionized the treatment of moderate-to-severe AD. JAK inhibitors are emerging in the therapeutic armamentarium of AD since inflammatory cytokines utilize the JAK/STAT pathway for downstream signal transduction. (Tsiogka et al., 2022) Nemolizumab, a monoclonal antibody that blocks the α subunit of the IL-31 receptor, rapidly alleviates itching by directly blocking signaling and is currently under investigation in AD (Serra-Baldrich et al., 2022). However, despite recent advances in AD treatment, achieving complete or near-complete response, a personalized approach for specific phenotypes, long-term disease control of signs and symptoms, on/off treatment regimens, and management of associated atopic comorbidities are still important unmet needs in patients with AD (Patrizi et al., 2022).

In this issue of *The Journal of Pharmacology and Experimental Therapeutics*, Nishimoto et al. report an interesting cellular model of human-induced pluripotent stem cell-derived keratinocyte-like cells for in vitro studies, a modern therapeutic approach for drug discovery and treatment, applied to AD.

In vitro trials or "clinical trials in a dish" may be considered a hot topic in the field of drug development as they allow researchers to test new compounds on human derived cells before planning human clinical trials (Ross and Wilson, 2014; Strauss and Blinova, 2017). These models, such as the pioneeristic study of Nishimoto and colleagues, address multiple needs of 21st century medicine (Strauss and Blinova, 2017). The use of these models beforehand may be crucial in identifying the best patient candidates to be enrolled in clinical trials, thereby increasing the likelihood of achieving the expected outcomes (Fermini et al., 2018a,b). For many diseases, physicians are now able to stratify patients according to clinical, laboratory, and imaging parameters, and the resulting patient stratification can be fundamental in decision-making. Furthermore, the use of in vitro trials would allow investigators to predict possible drug-related side effects/toxicities and prevent them in human patient studies (Ross and Wilson, 2014; Strauss and Blinova, 2017; Fermini et al., 2018b). The implementation of these models in clinical practice would also help in the prediction of response to a specific therapy, thus reducing the failure and containing the costs, especially in life-threatening diseases (i.e., cancer, infections, systemic immune diseases) (Fermini et al., 2018a,b; Strauss and Blinova, 2017).

Two main types of in vitro models are available: induced pluripotent stem cells (iPSC) and organ-on-a-chip (Strauss and Blinova, 2017). The iPSC-based model, the one applied in the paper by Nishimoto et al., relies on cellular reprogramming of skin and peripheral blood cells into pluripotent stem cells capable of transdifferentiating into a large variety of cell types (Grskovic et al., 2011; Costamagna et al., 2019; Rowe and Daley, 2019). This is the most recent and "close-to-reality" model that uses patient-derived tissue from unlimited and easy-to-retrieve sources (e.g., blood, adipose tissue, bone marrow), thus ensuring its wide application (Grskovic et al., 2011). In addition, iPSCs can be used as "bricks" to build 3D organ models, which could be considered quite a perfect setting for studies about disease pathogenesis and identifying new therapeutic targets (Strauss and Blinova, 2017; Costamagna et al., 2019). To this end, organs-on-chips have been developed over the past decade as devices to culture iPSCs (or other cell types) in continuously perfused microchambers designed to mimic tissue vasculature and complexity (Ma et al., 2021).

Address correspondence to: Roberto Giacomelli, Via Alvaro del Portillo 200, 00128 Rome, Italy. E-mail: r.giacomelli@policlinicocampus.it
dx.doi.org/10.1124/jpet.122.001469.

ABBREVIATIONS: AD, atopic dermatitis; iKera, keratinocytes; iPSC, induced pluripotent stem cells; TRP3, transient receptor potential member 3; TSLP, thymic stromal lymphopoietin.

The pathophysiology of AD involves a complex interaction of immune dysregulation, epidermal barrier dysfunction, altered skin microbiome, and IgE sensitization on a genetic background, triggered by environmental factors (Tsakok et al., 2019; Ständer, 2021). Current knowledge suggests that robust activation of Th2 (IL-4, IL-5, IL-13, IL-31) and Th22 (IL-22) immune responses in both skin and serum plays an important role in the immunopathogenesis of AD, especially in the acute phase, followed by a variable degree of Th1 (IFN-γ, TNF alpha) and Th17 (IL-17) activation in chronic disease. IL-4 and IL-13 are considered pivotal to the pathogenesis of AD and contribute significantly to the key signs and symptoms of the disease: erythema, lichenification, and pruritus. (Pappa et al., 2022; Ständer, 2021; Yosipovitch et al., 2019, 2020).

Pruritus is the most frequently reported parameter related to the clinical burden of AD, and its treatment is an important unmet need in AD patients (Misery et al., 2021; Fasseeh et al., 2022; Patrizi et al., 2022; Szöllősi et al., 2022). The cytokines produced by lymphocytes and keratinocytes in AD drive inflammation and act directly/indirectly as pruritogens by depolarizing pruriceptive nerve fibers. The main pruritogens involved in AD are histamine, Th2 cytokines (i.e., IL-4, IL-13, IL-31), thymic stromal lymphopoietin (TSLP), peptidases, and neuropeptides (Yosipovitch et al., 2020; Ständer, 2021). Itching in AD recruits several cellular players, including type C nerve fibers, lymphocytes, mast cells, and keratinocytes, consequently defining a complex neuroimmune axis (Yosipovitch et al., 2019, 2020). Together with neurons and inflammatory cells, epidermal keratinocytes contribute to AD-related pruritus in two different ways: directly through the secretion of different epithelial pruritogen (TSLP, IL33, neuropeptides) and indirectly through the production of an inefficient epidermal barrier characterized by increased transepidermal water loss, dry skin, and increased penetration of pruritogens in the stratum corneum (Sandilands et al., 2009; Tsakok et al., 2019; Szöllősi et al., 2022). Several receptors involved in pruritus are present on keratinocytes and are overexpressed in AD: PAR2, TSLPR, H1-H4 (Buhl et al., 2020; Zhao et al., 2020; Szöllősi et al., 2022). Among these receptors, PAR2 is particularly relevant to different aspects of AD pathogenesis as a driver of pruritus, barrier dysfunction, and inflammation. (Buhl et al., 2020; Zhao et al., 2020). Several exogenous (e.g., bacteria, allergens) and endogenous proteases can activate the G-coupled membrane receptor PAR2, which signals downstream via transient receptor potential subfamily V member 3 (TRPV3), a calcium-permeable cation channel abundantly expressed in cutaneous keratinocytes. TRPV3 is crucial to the production of neuronal pruritogen TSLP from keratinocytes and its inhibition abrogates PAR2 pruritus signaling (Hollenberg and Compton, 2002; Zhao et al., 2020). The relevance of the PAR2–TRPV3 signaling cascade in AD has been highlighted in other scientific papers, as overexpression of PAR2 in mice skin keratinocytes was sufficient to create a very confident model of human AD (Buhl et al., 2020).

Currently, thanks to advances in in vitro models, several cell lines are available to reproduce skin keratinocytes and to build an in vitro epidermal barrier from immortalized keratinocytes and primary keratinocytes (Liu et al., 2020; Rikken et al., 2020; Kim et al., 2022). On this basis, a skin-on-a-chip model, stimulated with specific concentrations of IL-4 and IL-13, induced cytokine secretion and expression of specific genes comparable to those observed in human AD skin (Kim et al., 2022). In addition, a vascularized skin model for AD using human keratinocytes, fibroblasts, pericytes, and endothelial cells derived from iPSCs has been published, showing the histopathologic features of the disease and has been successfully tested with dexamethasone and JAK inhibitors (Liu et al., 2020). This technology is evolving rapidly, and in the near future, it will be possible to reproduce a more sophisticated artificial tissue that includes iPSCs and neuroimmune cells involved in pruritogenesis during AD. Apart from skin disease modeling, iPSC-derived keratinocytes could have other relevant applications, for example, skin grafts for cutaneous wound healing and regenerative medicine (Zhong et al., 2021). To date, there is no skin model of AD consisting only of patient-derived iPSCs, whereas in vitro iPSC models of individual pathogenetic mechanisms (IL4/IL13 and PAR2) are now available (Kim et al., 2022).

The broadening understanding of AD pathogenesis has led to the development of novel therapeutic molecules (Chovatiya and Paller, 2021; Ständer, 2021). Monoclonal antibodies that inhibit the IL-4 and/or IL-13 pathway have revolutionized the treatment of moderate-to-severe AD. JAK inhibitors are emerging in the therapeutic armamentarium of AD since inflammatory cytokines use the JAK/STAT pathway for downstream signal transduction (Tsiogka et al., 2022). Nemolizumab, a monoclonal antibody that blocks the α subunit of the IL-31 receptor, rapidly alleviates itching by directly blocking signaling and is currently under investigation in AD (Serra-Baldrich et al., 2022).

In their breakthrough scientific paper, Nishimoto et al. demonstrate the different transdifferentiation steps of iPSCs in cutaneous keratinocytes (iKera), as confirmed by mRNA expression and immunofluorescence analysis. In this setting, iKera express the functional form of PAR2, as documented by intracellular calcium sparks upon treatment with PAR2 agonist peptides, similar to previous reports on pruritogenic PAR2–TRPV3 signal transduction in keratinocytes (Zhao et al., 2020). Interestingly, stimulation of iKera by PAR2 also led to the release of TNFα, suggesting the presence of a functional PAR2-mediated

proinflammatory response. An intriguing finding related to the different keratinocyte-related signaling pathways and their crosstalk in AD is the upregulation of PAR2 mRNA expression and corresponding protein levels after iKera treatment with IL-4. To corroborate this result, co-treatment of iKera with PAR2 agonist peptide plus IL-4/IL-13 further enhanced TSLP production compared with single stimulation. All these results support the crosstalk between the PAR2 and Th2 cytokine signaling cascades in the AD exacerbation cycle. Further investigation on PAR2 inhibitors is required to explore PAR2-targeted drugs as potential breakthroughs in AD treatment. In the future, it would be important for the commercial producers of iPSC to describe the gender origin of cells, due to the different expression of cellular pathways in male versus female.

In conclusion, following the findings of Nishimoto et al., iPSC-derived iKera appear to be a useful new tool to shed light on the pathogenesis of AD and facilitate the translation of preclinical findings into the clinical setting, at the dawn of the "clinical trial in a dish" era. This ongoing scientific research raises hope for a more effective and safer, personalized management of AD patients.

Giacomelli R and Fargnoli MC

Clinical and Research Unit of Rheumatology and Clinical Immunology, Fondazione Policlinico Campus Bio-Medico, Rome, Italy (R.G.); Rheumatology and Clinical Immunology, Department of Medicine, University Campus Bio-Medico, School of Medicine, Rome, Italy (R.G.); Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, School of Medicine, L'Aquila, Italy (M.C.F.); and Dermatology Unit, Ospedale San Salvatore, L'Aquila, Italy (M.C.F.)

Acknowledgments

The authors are indebted to Dr. Pietro Bearzi and Dr. Amelia Rigon for their kind assistance in the preparation of the manuscript.

References

Buhl T, Ikoma A, Kempkes C, Cevikbas F, Sulk M, Buddenkotte J, Akiyama T, Crumrine D, Camerer E, Carstens E, et al. (2020) Protease-activated receptor-2 regulates neuro-epidermal communication in atopic dermatitis. *Front Immunol* **11:**1740.
Chovatiya R and Paller AS (2021) JAK inhibitors in the treatment of atopic dermatitis. *J Allergy Clin Immunol* **148:**927–940.
Costamagna G, Andreoli L, Corti S, and Faravelli I (2019) iPSCs-based neural 3D systems: A multidimensional approach for disease modeling and drug discovery. *Cells* **8:**1438.
Fasseeh AN, Elezbawy B, Korra N, Tannira M, Dalle H, Aderian S, Abaza S, and Kaló Z (2022) Burden of atopic dermatitis in adults and adolescents: a systematic literature review. *Dermatol Ther (Heidelb)* **12:**2653–2668.
Fermini B, Coyne KP, and Coyne ST (2018a) Challenges in designing and executing clinical trials in a dish studies. *J Pharmacol Toxicol Methods* **94:**73–82.
Fermini B, Coyne, S T, and Coyne, K P (2018b). Clinical trials in a dish: a perspective on the coming revolution in drug development. *SLAS Discov* **23:**765–776.
Grskovic M, Javaherian A, Strulovici B, and Daley GQ (2011) Induced pluripotent stem cells–opportunities for disease modelling and drug discovery. *Nat Rev Drug Discov* **10:**915–929.
Hollenberg MD and Compton SJ (2002) International Union of Pharmacology. XXVIII. Proteinase-activated receptors. *Pharmacol Rev* **54:**203–217.
Kim K, Kim H, and Sung GY (2022) An interleukin-4 and interleukin-13 induced atopic dermatitis human skin equivalent model by a skin-on-a-chip. *Int J Mol Sci* **23:**2116.
Liu X, Michael S, Bharti K, Ferrer M, and Song MJ (2020) A biofabricated vascularized skin model of atopic dermatitis for preclinical studies. *Biofabrication* **12:**035002.
Ma C, Peng Y, Li H, and Chen W (2021) Organ-on-a-chip: a new paradigm for drug development. *Trends Pharmacol Sci* **42:**119–133.
Misery L, Brenaut E, Pierre O, Le Garrec R, Gouin O, Lebonvallet N, Abasq-Thomas C, Talagas M, Le Gall-Ianotto C, Besner-Morin C, et al. (2021) Chronic itch: emerging treatments following new research concepts. *Br J Pharmacol* **178:**4775–4791.
Pappa G, Sgouros D, Theodoropoulos K, Kanelleas A, Bozi E, Gregoriou S, Krasagakis K, and Katoulis AC (2022) The IL-4/-13 axis and its blocking in the treatment of atopic dermatitis. *J Clin Med* **11:**5633.
Patrizi A, Costanzo A, Patruno C, Busà VM, Chiricozzi A, and Girolomoni G (2022). Unmet needs in atopic dermatitis management: an expert consensus. *J Dermatolog Treat* **33:**2459–2465.
Rikken G, Niehues H, and van den Bogaard EH (2020) Organotypic 3D skin models: human epidermal equivalent cultures from primary keratinocytes and immortalized keratinocyte cell lines. *Methods Mol Biol* **2154:**45–61.
Ross NT and Wilson CJ (2014) In vitro clinical trials: the future of cell-based profiling. *Front Pharmacol* **5:**121.
Rowe RG and Daley GQ (2019) Induced pluripotent stem cells in disease modelling and drug discovery. *Nat Rev Genet* **20:**377–388.
Sandilands A, Sutherland C, Irvine AD, and McLean WHI (2009) Filaggrin in the frontline: role in skin barrier function and disease. *J Cell Sci* **122:**1285–1294.
Serra-Baldrich E, Santamaría-Babí LF, and Francisco Silvestre J (2022) Nemolizumab: an innovative biologic treatment to control interleukin 31, a key mediator in atopic dermatitis and prurigo nodularis. *Actas Dermosifiliogr* **113:**674–684.
Ständer S (2021) Atopic dermatitis. *N Engl J Med* **384:**1136–1143.33761208.
Strauss DG and Blinova K (2017) Clinical trials in a dish. *Trends Pharmacol Sci* **38:**4–7.
Szöllősi AG, Oláh A, Lisztes E, Griger Z, and Tóth BI (2022). Pruritus: a sensory symptom generated in cutaneous immuno-neuronal crosstalk. *Front Pharmacol* **13:**745658.
Tsakok T, Woolf R, Smith CH, Weidinger S, and Flohr C (2019) Atopic dermatitis: the skin barrier and beyond. *Br J Dermatol* **180:**464–474.
Tsiogka A, Kyriazopoulou M, Kontochristopoulos G, Nicolaidou E, Stratigos A, Rigopoulos D, and Gregoriou S (2022) The JAK/STAT pathway and its selective inhibition in the treatment of atopic dermatitis: a systematic review. *J Clin Med* **11:**4431.
Yosipovitch G, Berger T, and Fassett MS (2020). Neuroimmune interactions in chronic itch of atopic dermatitis. *J Eur Acad Dermatol Venereol* **34:**239–250.
Yosipovitch G, Misery L, Proksch E, Metz M, Ständer S, and Schmelz M (2019) Skin barrier damage and itch: review of mechanisms, topical management and future directions. *Acta Derm Venereol* **99:**1201–1209.
Zhao J, Munanairi A, Liu XY, Zhang J, Hu L, Hu M, Bu D, Liu L, Xie Z, Kim BS, et al. (2020) PAR2 mediates itch via TRPV3 signaling in keratinocytes. *J Invest Dermatol* **140:**1524–1532.
Zhong J, Wang H, Yang K, Wang H, Duan C, Ni N, An L, Luo Y, Zhao P, Gou Y, et al. (2021) Reversibly immortalized keratinocytes (iKera) facilitate re-epithelization and skin wound healing: Potential applications in cell-based skin tissue engineering. *Bioact Mater* **9:**523–540.