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Published in:
Experimental dermatology

DOI:
10.1111/exd.14648

Publication date:
2022

Received: 14 March 2022 | Revised: 13 July 2022 | Accepted: 15 July 2022

DOI: 10.1111/exd.14648

REVIEW ARTICLE

Experimental Dermatology WILEY

Cutting edge technologies in chronic inflammation research

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Abstract

This concise review provides the broad background and selection from the literature for a Keynote lecture at EHSF 2022 on state of the art technologies in inflammation research, with an emphasis on disease of the skin and the nervous system. The value of ex vivo skin explant models is discussed, as well as the innovative use of animal models, wherein the crucial roles of antigen experience and "wild" microbiota are emphasized. Spectral flow cytometry allowing large surface marker panels to be explored is touched upon, as well as multiplex technology for cytokines and other analytes important for inflammation and tissue damage. Single-cell sequencing and in situ transcriptomics (spatial profiling) now provide exciting granular information on functional cell subsets, interactions and plasticity. A selection of novel research and diagnostic tools for antibodies against linear peptides or gangliosides is presented. Finally, the review discusses a new anti-inflammatory strategy against skin inflammation with a panel of protease inhibitors derived from the protein fraction of industrial starch potatoes.

KEYWORDS
animal model, diagnostic tools, ex vivo model, gut microbiota, inflammation, nervous system, protease inhibitors, single-cell sequencing, skin, spatial profiling, spectral flow cytometry

1 | INTRODUCTION

As elegantly reviewed by McInnes and Gravallese,[1] in the past two decades, the toolbox of biologicals to treat immune-mediated inflammatory diseases (IMID) has been transformed significantly. The extensive experience from clinical trials and routine patient use of biologicals against cytokine–receptor systems has allowed to build a molecular taxonomy of disease hierarchies. Herein, cytokine hubs can be identified,[2] with for example IL-23 and IL-17A playing central roles in psoriatic arthritis.

The target tissue itself is orchestrating disease and resolution with its stromal cell types interacting with immune cell subsets. Leading examples include tumour necrosis factor (TNF)-α neutralization in rheumatoid arthritis, unexpected benefits from B-cell depletion in multiple sclerosis, and contrasting efficacy of TNF-α and IL-23 inhibitors versus IL-17A inhibitors in Crohn's disease.

To further accelerate translational IMID research requires detailed understanding of complex (and sometimes perplexing) immunological pathways, hand in hand with implementation of advanced technologies. Multi-omics technologies rely on close collaboration between clinician-investigators, immunologist, molecular biologists and bioinformaticians.

This concise review provides an accessible overview of selected novel technologies. In vignette style, the review will draw on prominent recent literature and examples from own joint work, with a focus on inflammatory skin and nervous system diseases.

The continued crucial role of in vivo and ex vivo model innovation is argued based on the imiquimod mouse model for psoriasis lesion development, human skin explant culture and marmoset monkey EAE (experimental autoimmune encephalomyelitis) modelling multiple sclerosis.[3]

Examples of single-cell transcriptomics of skin and human fetal microglia are discussed. In situ transcriptomics provides novel

insights into functional skin cell subsets within the original lesion context such as in psoriasis and hidradenitis suppurativa.

Highly sensitive immuno-polymerase chain reaction (PCR) for large panels of inflammatory analytes promotes mechanistic pathway analyses in patients and model systems. Translocation of microbiota compounds across skin and gut epithelium is critical to inflammation and therapy, and novel technology is described. Combinatorial chemistry allows decoding of antibody responses not only against classic peptide epitopes but also against glycopeptides such as multiplexed carbohydrates in the Guillain–Barré syndrome.

Finally, a novel therapy against skin inflammation driven by gut proteolytic enzymes is presented, wherein a panel of protease inhibitors from starch potato provides clinical benefit for patients with stoma or Hirschsprung's disease.

2 | WHOLE HUMAN SKIN EXPLANT FUNCTIONAL CULTURES

Since plastic surgeons routinely excise healthy human skin in for instance breast and abdominal reduction procedures, there is an ample and continuous supply of skin for explant functional cultures. The group of E.P. Prens (Erasmus MC, Rotterdam, the Netherlands) has developed a relatively affordable and straightforward system to culture full thickness punch biopsies of healthy donor and patients.[4] In the membrane of commercially available Transwell culture plates, a small hole is made and the punch biopsy is tightly positioned such that the epidermis is at the gas interface, while the dermis is in the culture media. This multi-day culture system hence allows manipulations such as addition of cytokines or (therapeutic) antibodies to the medium. Also, the gas interface can be modulated simply by placing the Transwell culture plate into an airtight plastic bag with a valve, for example to manipulate oxygen pressure. Readouts include the culture supernatant since the biopsy secretes bioactive compounds, as well as in situ analysis of the whole biopsy, or cell subset isolation by fluorescence activated cell sorting (FACS). As an example, we have used this system to demonstrate that agonist anti-CD40 antibody induces cytokine production through IL-1.[5] Combinations of this culture system with in situ analysis and with skin cell suspensions were used to investigate hidradenitis suppurative immunopathology and to identify new cytokine targets for treatment.[6,7]

3 | MULTIPLEX ANALYSES OF LEUCOCYTE SUBSETS AND INFLAMMATORY COMPOUNDS

When using peripheral blood as a source for leucocyte subsets, it is important to be fully aware that the much used peripheral blood mononuclear cells (PBMC) lack the most numerous population, that is the neutrophils. In a most useful concise review, the technical variation between PBMC and whole blood in cytokine production was discussed.[8] Also, features of functional assays with whole blood versus PBMC were summarized.[8] Most interestingly, with innovations in flow cytometry/FACS and multiplex protein assays, in as little as 100 µl of blood for instance from newborns, around 50 leucocyte subsets and hundreds of cytokines and other analytes of interest can be determined.[9]

Spectral flow cytometry based on the use of several detectors per fluorochrome instead of one, 40 or more labelled antibodies can now be used. This expands the possibilities for leucocyte typing enormously.

A very successful multiplex technology for cytokines and other analytes in blood and other fluids is from the company Olink (Uppsala, Sweden). Detection relies on joint use of two monoclonal antibodies against distinct epitopes on the analyte of interest, for instance a cytokine or a molecule reflecting tissue damage. Both antibodies have a DNA tail attached, and only when both antibodies bind can the DNA align. This allows subsequent detection by microfluidic quantitative PCR. This approach combines the highly specific binding of monoclonal antibodies with the high sensitivity of PCR. For a single-patient sample, multiplex assays in formats of 48, 96 or 384 analytes can be performed. Preselected panels of analytes are offered, for instance focusing on inflammation, oncology or neurological markers. This technology has been used very recently for hidradenitis suppurativa.[10] Proteomic signatures, including Olink technology, have been reviewed for atopic dermatitis.[11]

4 | SINGLE-CELL SEQUENCING AND IN SITU SPATIAL TRANSCRIPTOMICS

Assessing the full transcriptome of individual cells in an unbiased manner has revolutionized biology and pathology. From any type of tissue of interest, single cells can be isolated by FACS, followed by technically challenging but robust RNA sequencing. For instance, developmental trajectories of fetal human microglia were established.[12] In parallel, the technical journal *Nature Methods* designated in situ spatially resolved transcriptomics as the Method of the Year 2021.[13] Some prime examples for skin diseases include the finding that developmental cell programs are co-opted during inflammation,[14] and that anatomically distinct fibroblast subsets determine skin autoimmune patterns in vitiligo in patients and mouse models.[15] A most recent comprehensive review extensively explains principles and technologies to integrate single-cell and spatial transcriptomics, which jointly can elucidate cellular and molecular pathways in tissue dynamics during health and disease.[16]

5 | NOVEL ANTIBODY ASSAYS WITH LINEAR PEPTIDE ARRAYS AND GANGLIOSIDES

New methods for peptide synthesis plus transfer now allow large numbers (up to thousands) of linear amino acid sequences to be spotted onto a single glass slide. The slide can then be probed with

patient samples to detect antibodies, for instance against pathogen antigens for vaccine design and immune-therapeutics, and for self-antigens in autoimmune disease.[17–19] Current efforts include synthesis of glycans plus peptides, in particular for bacterial cell wall peptidoglycan (e.g.[20]), such that also antibodies directed against glycans or combined peptide/glycan epitopes can be detected. Peptidoglycan translocation over the gut wall is an important feature of gut permeability, and peptidoglycan can co-drive inflammation in arthritic joints and in the brain.[21]

Chemoenzymatic synthesis methods allow to create the lipo-oligosaccharide (LOS) core domains of *Campylobacter jejuni*, which mimic human gangliosides. Upon infection and enteritis, in some patients the antibodies do not only act against *C. jejuni*, but also cross react with gangliosides in human peripheral nerves, leading to paralytic disease (molecular mimicry).[22] The synthesis of glycan arrays now enables the fine-distinction between antibodies binding to closely related gangliosides and LOS.[23]

6 | INNOVATIVE ANIMAL MODELS—PATHOGEN EXPERIENCE AND "WILD" MICROBIOTA

6.1 | Value of animal models

In several European countries, there is a strong societal and political drive against animal models, which oftentimes is scientifically poorly informed and has been euphemistically dubbed "experimental animal-free innovation" in The Netherlands. However, it is of paramount importance that highly complex inflammatory disease with interplay of gut microbiota, the mobile immune system and target organs are exceedingly difficult to mimic in vitro. The COVID-19 pandemic has once again underscored the great value and acute importance of animal models, such as in hamsters and rhesus macaques.[24] For instance, progress in immunopathology and drug innovation for hidradenitis suppurativa is hampered by the lack of suitable small animal models (e.g.[25]).

6.2 | Imiquimod mouse model

Guided by observing psoriasis lesions at sites distant from local application of imiquimod (Aldara® cream, against basal cell carcinoma and genital warts), the group of E.P. Prens conceived a new mouse model for development of psoriasis-like lesions in mice.[26] Simply painting the clinical Aldara® cream onto the shaven back of wildtype inbred laboratory mice induces prominent lesion features within 3–6 days, including skin redness, scaling and thickening. This model has become internationally widely used for studies on pathogenesis and treatment. Ward and colleagues have rightly called attention to proper execution and interpretation of the model.[27] In addition, we have provided a comprehensive overview of the literature and extensive practical guidance how to avoid pitfalls in animal inflammation models.[28]

6.3 | Improving models by replacing complete Freund's Adjuvant (CFA)

Many animal models for inflammatory and autoimmune disease and rely on the use of a strong adjuvant containing heat-killed *Mycobacterium tuberculosis* particles, called CFA. This adjuvant is not allowed for use in humans since it generates granulomas and serious discomfort. The late influential immunologist Charles Janeway called CFA "the immunologist's dirty little secret" since it is required to break T- and B-cell tolerance in healthy organisms.[29] In fact, a case report of accidental self-injection with CFA suggests that this adjuvant induces polyautoreactivity.[30]

We have demonstrated that CFA can be replaced by Incomplete Freund's Adjuvant (IFA), which contains no *M. tuberculosis*, for the induction of multiple sclerosis (MS)-like disease in marmoset monkeys (*Callithrix jacchus*).[31–33] We argue that this is important for three reasons: (a) reduction of animal discomfort; (b) improvement of the preclinical value due to a strongly reduced cytokine storm; and (c) disproving the highly prevalent dogma that microbial compounds must necessarily be co-administered with the self-antigen to break tolerance. It is quite likely that breaking tolerance with myelin self-antigen in IFA is effective in the marmosets since they grow up in a conventional environment allowing them to build up pathogen-experience for at least a full year.

6.4 | Pathogen experience and wild microbiota

Finally, in a most important broad development, work with wild mice and pet shop mice demonstrates the crucial importance of pathogen experience, that is the exposure to a wide array of pathogens over a protracted period in a physiological setting to more adequately mimic the human immune system[34] (review by Graham[35]). Previously, similar approaches had been applied for wild rats.[36–38] The standard commercial inbred mouse or rat lives in very clean animal facilities under a specified pathogen free (SPF) regime (typically screened for a list of 20–50 pathogens). Furthermore, animals are often used at young age, from 8 to 12 weeks onwards. Hence, these animals have very limited pathogen and antigen experience, limiting innate immune mechanisms, development of their T- and B-cell repertoire and establishment of T-effector memory populations (Tem) in different tissues such as the skin (e.g.[39]).

Innovative applications of the insight that pathogen exposure and wild microbiota rend mice immunologically much more similar to humans, include deliberate exposure to a selection of pathogens,[40] and elegant creation of "wildlings" where wild microbiota is introduced into the gut of inbred mice.[41] For instance, it was shown that

mice can be protected against diet-induced obesity by neonatal exposure to a "wild" microbiota.[42]

Using the EAE model in adult (over 1 year) outbred marmoset monkeys, we demonstrated that a dietary intervention limited disease and inflammation of the spinal cord.[43] For the first time the gut microbiota of the marmoset was typed, and we subsequently analysed the evolution of its composition along EAE time course from a gut ecology perspective.[44]

Overall, these novel insights provide ample arguments that immunity in animal models can very accurately reflect many mechanisms of human immunity. Hence, well-controlled innovative animal models will remain vital to rapid progress in research on immunopathology, diagnostics and therapeutic strategies.

7 | LIMITING SKIN INFLAMMATION WITH A PANEL OF PROTEASE INHIBITORS

At sites where the skin is repeatedly or chronically exposed to proteolytic enzymes of the gut content, painful and severe inflammation of the damaged skin can develop, such as in stoma patients, infants with Hirschsprung's disease and healthy babies with diaper dermatitis. Since a wide array of pancreatic and gut enzymes contribute to physiologic proteolysis, we reasoned that a panel of protease inhibitors should prove effective to prevent and treat skin inflammation. Industrial starch potatoes have a minor protein fraction of 1%–2%, which mainly consists of a panel of 25 very well-characterized protease inhibitors.[45] Proof of this concept was provided by exposing back skin of healthy volunteers to a mix of commercially available proteases, and to patient ileostomy fluid, showing that the protease inhibitors effectively inhibit skin inflammation.[46]

Rewardingly, this concept was independently confirmed and extended to successful treatment of four infants with Hirschsprung's disease.[47] This concept has now reached patient/parent-customer as a commercial cosmetic cream on a cetomacrogol basis (note: academic investigators involved have no commercial interests in this product). Currently, this concept is applied anecdotically also to patients with other inflammatory skin diseases, as summarized in Table 1.

8 | CONCLUDING REMARKS

It is hoped that this review provides ample suggestions and directions for further development and applications of novel technologies for research into inflammatory diseases, notably of the skin. An extensive literature list is provided both to primary papers and to excellent detailed reviews for several of the technologies discussed. There is little doubt that such new technologies in conjunction with the large number of new biologicals in clinical trials will accelerate progress, ultimately benefiting patients by improved diagnosis, therapy and therapy monitoring by companion diagnostics.

TABLE 1 Current applications of potato protease inhibitors against skin inflammation

Healthy babies with normal or severe diaper rash—General population, driven by parent-customers
Infants with Hirschsprung's disease—Driven by paediatric surgeons in the Netherlands (ongoing clinical trial) and Switzerland.[47]
Patients with idiopathic perinasal dermatitis—Single-user anecdotal evidence
Adult patients with stoma—Driven by dermatologists and Dutch patient groups
Patients with genetic blistering disease epidermolysis bullosa (chronic wounds)—Anecdotal evidence

AUTHOR CONTRIBUTIONS

Jon D. Laman conceptually developed and wrote this review.

ACKNOWLEDGEMENTS

The author thanks several colleagues for longstanding collaborations and productive discussions, some of which are directly reflected in this review: Johanneke van Embden, Bert't Hart, Peter Heeringa, Barbara Horvath, Marc Laus, Leo van Lieshout, Felix Löffler, Joost Meijer, William Parker and Errol P. Prens.

CONFLICT OF INTEREST

The author has no conflicts of interest related to the content of the manuscript.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

REFERENCES

1. McInnes IB, Gravallese EM. Immune-mediated inflammatory disease therapeutics: past, present and future. *Nat Rev Immunol*. 2021;21:680-686.
2. Schett G, McInnes IB, Neurath MF. Reframing immune-mediated inflammatory diseases through signature cytokine hubs. *N Engl J Med*. 2021;385:628-639.
3. 't Hart BA, Laman JD, Kap YS. Merits and complexities of modeling multiple sclerosis in non-human primates: implications for drug discovery. *Expert Opin Drug Discov*. 2018;13:387-397.
4. Companjen AR, van der Wel LI, Wei L, Laman JD, Prens EP. A modified *ex vivo* skin organ culture system for functional studies. *Arch Dermatol Res*. 2001;293:184-190.
5. Companjen AR, van der Wel LI, Boon L, Prens EP, Laman JD. CD40 ligation-induced cytokine production in human skin explants is partly mediated via IL-1. *Int Immunol*. 2002;14:669-676.
6. van der Zee HH, de Ruiter L, Boer J, et al. Alterations in leucocyte subsets and histomorphology in normal-appearing perilesional skin and early and chronic hidradenitis suppurativa lesions. *Br J Dermatol*. 2012;166:98-106.
7. van der Zee HH, Laman JD, de Ruiter L, Dik WA, Prens EP. Adalimumab (antitumour necrosis factor-alpha) treatment of hidradenitis suppurativa ameliorates skin inflammation: an in situ and ex vivo study. *Br J Dermatol*. 2012;166:298-305.
8. Brodin P, Duffy D, Quintana-Murci L. A call for blood-in human immunology. *Immunity*. 2019;50:1335-1336.
9. Olin A, Henckel E, Chen Y, et al. Stereotypic immune system development in newborn children. *Cell*. 2018;174:1277-92.e14.

10. Navrazhina K, Frew JW, Grand D, et al. IL-17RA blockade by brodalumab decreases inflammatory pathways in hidradenitis suppurativa skin and serum. *Br J Dermatol*. 2022. doi:10.1111/bjd.21060. Online ahead of print.
11. Mikhaylov D, Del Duca E, Guttman-Yassky E. Proteomic signatures of inflammatory skin diseases: a focus on atopic dermatitis. *Expert Rev Proteomics*. 2021;18:345-361.
12. Kracht L, Borggrewe M, Eskandar S, et al. Human fetal microglia acquire homeostatic immune-sensing properties early in development. *Science*. 2020;369:530-537.
13. Method of the year 2020: spatially resolved transcriptomics. *Nat Methods*. 2021;18:1.
14. Reynolds G, Vegh P, Fletcher J, et al. Developmental cell programs are co-opted in inflammatory skin disease. *Science*. 2021;371:eaba6500.
15. Xu Z, Chen D, Hu Y, et al. Anatomically distinct fibroblast subsets determine skin autoimmune patterns. *Nature*. 2022;601:118-124.
16. Longo SK, Guo MG, Ji AL, Khavari PA. Integrating single-cell and spatial transcriptomics to elucidate intercellular tissue dynamics. *Nat Rev Genet*. 2021;22:627-644.
17. Loeffler FF, Foertsch TC, Popov R, et al. High-flexibility combinatorial peptide synthesis with laser-based transfer of monomers in solid matrix material. *Nat Commun*. 2016;7:11844.
18. Heiss K, Heidepriem J, Fischer N, et al. Rapid response to pandemic threats: immunogenic epitope detection of pandemic pathogens for diagnostics and vaccine development using peptide microarrays. *J Proteome Res*. 2020;19:4339-4354.
19. Jaenisch T, Heiss K, Fischer N, et al. High-density peptide arrays help to identify linear immunogenic B-cell epitopes in individuals naturally exposed to malaria infection. *Mol Cell Proteomics*. 2019;18:642-656.
20. Dallabernardina P, Benazzi V, Laman JD, Seeberger PH, Loeffler FF. Automated glycan assembly of peptidoglycan backbone fragments. *Org Biomol Chem*. 2021;19:9829-9832.
21. Laman JD, 't Hart BA, Power C, Dziarski R. Bacterial peptidoglycan as a driver of chronic brain inflammation. *Trends Mol Med*. 2020;26:670-682.
22. Laman JD, Huizinga R, Boons GJ, Jacobs BC. Guillain-Barré syndrome: expanding the concept of molecular mimicry. *Trends Immunol*. 2022;43:322-330.
23. Li T, Wolfert MA, Wei N, Huizinga R, Jacobs BC, Boons GJ. Chemoenzymatic synthesis of *campylobacter jejuni* lipo-oligosaccharide core domains to examine Guillain-Barré syndrome serum antibody specificities. *J Am Chem Soc*. 2020;142:19611-19621.
24. Genzel L, Adan R, Berns A, et al. How the COVID-19 pandemic highlights the necessity of animal research. *Curr Biol*. 2020;30:4328.
25. van der Zee HH, Laman JD, Prens EP. Can animal skin diseases or current transgenic mice serve as a model for hidradenitis suppurativa? *Dermatology*. 2012;225:9-13.
26. van der Fits L, Mourits S, Voerman JS, et al. Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis. *J Immunol*. 2009;182:5836-5845.
27. Hawkes JE, Gudjonsson JE, Ward NL. The snowballing literature on imiquimod-induced skin inflammation in mice: a critical appraisal. *J Invest Dermatol*. 2017;137:546-549.
28. Laman JD, Kooistra SM, Clausen BE. Reproducibility issues: avoiding pitfalls in animal inflammation models. *Methods Mol Biol*. 2017;1559:1-17.
29. Greslehner GP. The vaccinologist's "dirty little secret": a better understanding of structure-function relationships of viral immunogens might advance rational HIV vaccine design. *Arch Virol*. 2021;166:1297-1303.
30. Watad A, Bragazzi NL, Shoenfeld Y. Immunologist's little dirty secret finger: a case report of polyautoimmunity following an accidental self-injection of complete Freund's adjuvant. *Isr Med Assoc J*. 2020;22:393-394.
31. Jagessar SA, Kap YS, Heijmans N, et al. Induction of progressive demyelinating autoimmune encephalomyelitis in common marmoset monkeys using MOG34-56 peptide in incomplete freund adjuvant. *J Neuropathol Exp Neurol*. 2010;69:372-385.
32. Jagessar SA, Heijmans N, Blezer EL, et al. Unravelling the T-cell-mediated autoimmune attack on CNS myelin in a new primate EAE model induced with MOG34-56 peptide in incomplete adjuvant. *Eur J Immunol*. 2012;42:217-227.
33. Haanstra KG, Jagessar SA, Bauchet AL, et al. Induction of experimental autoimmune encephalomyelitis with recombinant human myelin oligodendrocyte glycoprotein in incomplete Freund's adjuvant in three non-human primate species. *J Neuroimmune Pharmacol*. 2013;8:1251-1264.
34. Beura LK, Hamilton SE, Bi K, et al. Normalizing the environment recapitulates adult human immune traits in laboratory mice. *Nature*. 2016;532:512-516.
35. Graham AL. Naturalizing mouse models for immunology. *Nat Immunol*. 2021;22:111-117.
36. Devalapalli AP, Lesher A, Shieh K, et al. Increased levels of IgE and autoreactive, polyreactive IgG in wild rodents: implications for the hygiene hypothesis. *Scand J Immunol*. 2006;64:125-136.
37. Lesher A, Li B, Whitt P, et al. Increased IL-4 production and attenuated proliferative and pro-inflammatory responses of splenocytes from wild-caught rats (*Rattus norvegicus*). *Immunol Cell Biol*. 2006;84:374-382.
38. Ren D, Beinart D, Pi C, et al. Returning to the wild: restoring natural levels of immune function to laboratory animals. *J Appl Life Sci Int*. 2017;14:1-13.
39. Linehan JL, Harrison OJ, Han SJ, et al. Non-classical immunity controls microbiota impact on skin immunity and tissue repair. *Cell*. 2018;172:784-796.
40. Reese TA, Bi K, Kambal A, et al. Sequential infection with common pathogens promotes human-like immune gene expression and altered vaccine response. *Cell Host Microbe*. 2016;19:713-719.
41. Rosshart SP, Herz J, Vassallo BG, et al. Laboratory mice born to wild mice have natural microbiota and model human immune responses. *Science*. 2019;365:eaaw4361.
42. Hild B, Dreier MS, Oh JH, et al. Neonatal exposure to a wild-derived microbiome protects mice against diet-induced obesity. *Nat Metab*. 2021;3:1042-1057.
43. Kap YS, Bus-Spoor C, van Driel N, et al. Targeted diet modification reduces multiple sclerosis-like disease in adult marmoset monkeys from an outbred colony. *J Immunol*. 2018;201:3229-3243.
44. Perez-Muñoz ME, Sugden S, HJM H, t Hart BA, Laman JD, Walter J. Nutritional and ecological perspectives of the interrelationships between diet and the gut microbiome in multiple sclerosis: insights from marmosets. *iScience*. 2021;24:102709.
45. Pouvreau L, Gruppen H, Piersma SR, van den Broek LA, van Koningsveld GA, Voragen AG. Relative abundance and inhibitory distribution of protease inhibitors in potato juice from cv. Elkana. *J Agric Food Chem*. 2001;49:2864-2874.
46. Ruseler-van Embden JG, van Lieshout LM, Smits SA, van Kessel I, Laman JD. Potato tuber proteins efficiently inhibit human faecal proteolytic activity: implications for treatment of perianal dermatitis. *Eur J Clin Invest*. 2004;34:303-311.
47. Berger S, Rufener J, Klimek P, Zachariou Z, Boillat C. Effects of potato-derived protease inhibitors on perianal dermatitis after colon resection for long-segment Hirschsprung's disease. *World J Pediatr*. 2012;8:173-176.

How to cite this article: Laman JD. Cutting edge technologies in chronic inflammation research. *Exp Dermatol*. 2022;31(Suppl. 1):17-21. doi: 10.1111/exd.14648