Introduction
Owing to its exteriority, the skin has captivated the human imagination since ancient Roman and Egyptian civilizations. Yet, modern-day experimental dermatology and immunology did not take root until the late 19th and 20th centuries, respectively. In parallel, experimental dermatologists and immunologists studied contact hypersensitivity, graft rejection and histocompatibility, and adjuvant responses (Chase, 1985). These foundational works revealed the immune underpinnings of skin diseases while still viewing the skin as an epithelial barrier that recruited immune allies only under duress.

Advancing technologies illuminated the myriad of immune cells that reside in and continually patrol the skin, shifting the view that the skin is simply an inert barrier (Kobayashi et al., 2019a). Inspired by the 2019 Montagna Biology of Skin Symposium, we discuss the remarkable discoveries in skin immunity that have resulted from imaging, tissue processing, and genomic techniques (Figure 1). We also highlight the importance of these tools in understanding immune dysfunction in inflammatory skin diseases. Finally, we explore emerging technologies and their potential for further expanding the knowledge of skin immunity.

Seeing is believing. In 1868, Paul Langerhans, enabled by rudimentary light microscopy, uncovered cells with dendrites in the epidermis (Langerhans, 1868), which he concluded were epidermal nerves. His discovery of Langerhans cells (LCs) was the first known observation of immune cells in normal skin. In 1949, Andrews and Andrews distinguished lymphocytes in normal epidermis using light microscopy (Andrew and Andrew, 1949). A few decades later, Streilein (1978) built upon these and other works to propose that the skin had a dedicated immune component, which he termed skin-associated lymphoid tissue.

Since then, sophisticated imaging techniques, most notably fluorescence microscopy, have been widely used to illuminate the immune microanatomy of the skin (Kabashima et al., 2019). Fluorescence microscopy enabled the simultaneous visualization of multiple cell types and their expressed factors at a higher resolution than simple light microscopy (Sanderson et al., 2014). Initially used to detect epidermal-resident dendritic epidermal T cells (DETCs) and LCs (Havran et al., 2018; Steiner et al., 1988), microscopic analyses have revealed the tightly controlled spatial distribution of the myriad of immune cells in the skin (Kabashima et al., 2019). LCs and intraepithelial lymphocytes (DETCs [Havran and Allison, 1990] CD8+ resident memory T cells [T_em] [Schenkel and Masopust, 2014]) and innate lymphoid cells (ILCs) (Kobayashi et al., 2019b) are capable of traversing the basement membrane reside in the epidermis. The upper dermis houses several dendritic cells (DCs) subsets (Tamoutounour et al., 2013), γδ T cells (Gray et al., 2011), CD4 T helper (Adachi et al., 2015), and regulatory T cells (Ali et al., 2017) and ILCs (Kobayashi et al., 2019b). These cells are enriched around hair follicles (Adachi et al., 2015), highlighting that this region is a key immunological hub in the skin. The lower dermis houses various macrophage subsets in close apposition to vasculature, nerves, and adipocytes (Silva et al., 2019). In addition to immune localization, dynamic imaging has divulged immune surveillance function in the normal skin. Images of LCs extending their dendrites through the epidermis captured their homeostatic uptake of external antigens (Ouchi et al., 2011). LCs and other DCs migrate to the

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The skin’s physical barrier is reinforced by an arsenal of immune cells that actively patrol the tissue and respond swiftly to penetrating microbes, noxious agents, and injurious stimuli. When unchecked, these same immune cells drive diseases such as psoriasis, atopic dermatitis, and alopecia. Rapidly advancing microscopy, animal modeling, and genomic and computational technologies have illuminated the complexity of the cutaneous immune cells and their functions in maintaining skin health and driving diseases. Here, we discuss the recent technology-driven breakthroughs that have transformed our understanding of skin immunity and highlight burgeoning areas that hold great promise for future discoveries.

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Abbreviations: 3D, three-dimensional; CyTOF, mass cytometry; DC, dendritic cell; DETC, dendritic epidermal T cell; ECM, extracellular matrix; ILC, innate lymphoid cell; LC, Langerhans cell; scRNAseq, single-cell RNA sequencing; T_em, resident memory T cell

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lymph nodes to induce T effector and reg-
ulatory cells and/or provide homeostatic
signals to maintain these populations in the
normal skin (Naik et al., 2015; Seneschal
et al., 2012).

Pioneered in 1990, multiphoton mi-
scropy allowed for deeper tissue
penetration and opened the door to intravital imaging (Denk et al., 1990).
Coupled with the generation of fluores-
cence reporter animals, multiphoton im-
aging was used to view live image
immune cells (Kabashima and Egawa,
2014). This enabled the three-
dimensional (3D) reconstruction of im-
mune niches and revealed the interaction
of leukocytes with the skin’s structural
components (Kabashima and Egawa,
2014). Intravital imaging is also a power-
ful tool to visualize the induction and
propagation of inflammatory responses
(Obeidy et al., 2018). A key feature of
inflammatory responses in the skin is a
compromised barrier, especially in the
case of infectious agents or tissue injury.
Live imaging identified neutrophils as first
responders infiltrating within hours of
epidermal breach (Obeidy et al., 2018;
Peters et al., 2008) and the kinetics of
DC migration to the lymph nodes under
stress, illustrating that functionally
specialized DC subsets migrate with
specific kinetics to induce adaptive re-
ponses (Tamoutounour et al., 2013).

Quantitative imaging combined with
pathways-specific modulation of
cell–cell interactions, cell–extracellular
matrix (ECM) interactions, or motility
has unearthed therapeutic targets in
inflammation (Matheu et al., 2008;
Overstreet et al., 2013). For instance,
perivascular lymphocytes and DCs form
clusters in an IL-1R–dependent manner
to drive contact dermatitis (Natsuaki
et al., 2014). Imaging studies provided
insight into how innate and adaptive im-
mune cells control skin tumors. To this
end, a role for CD8
++ T
RM and innate
immune cells in restraining melanoma and
epithelial neoplasms has been identified
(Caulin et al., 2007; Park et al., 2019).
Thus, imaging techniques have provided
invaluable insights into the location,
migration, interactions, and functions of
immune cells in skin health and disease.

**Cytometry and genomic technologies widen the lens.** Perhaps the most un-
derappreciated and widely used method-
ology in skin immunology is the ability to
efficiently extract viable cells from the
skin while preserving the expression of
surface proteins for phenotypic analysis.
This was first accomplished by employing
a serine protease, trypsin, to digest ECM
and severe cell–cell interactions to
obtain DETCs and LCs (Havran and
Allison, 1990; Steiner et al., 1988).
Since then, sophisticated enzymes with
minimal nonspecific activity have
become available and are used to prepare
cell suspensions for a number of down-
stream analysis platforms (Botting et al.,
2017; Clark et al., 2006).

Flow cytometry has been the corner-
stone of immunology for many decades
and is ubiquitously used to analyze cells
from healthy and diseased skin (Adan
et al., 2017). Antibodies raised to specific
protein moieties (surface markers, cyto-
kines, transcription factors, and signaling
components) are coupled with fluorescent
indicators and have empowered re-
searchers to examine multiple cellular
parameters simultaneously and quantita-
tively. In 1969, Herzenberg published a
new technique to obtain highly purified
cell populations, called FACS (Hulett et al.,
1969). Cells purified directly from the skin
with FACS have been used for functional
in vitro studies (Seneschal et al., 2012),
in vivo cell transfer experiments (Schenkel
and Masopust, 2014), and downstream
tissue and cell-specific genomic analysis
(Cheng et al., 2018).

However, fluorescent indicators have
restricted analysis to the visible-light and
UV spectrum and limited the number of
parameters that could be measured
simultaneously. In 2009, Bandura et al.,
(2009) overcame these limitations by
developing mass cytometry (CyTOF).
CyTOF blends flow cytometry with mass
spectrometry using metal-conjugated an-
tibodies to dramatically increase the
number of analytes from as few as 10,000
cells, enabling efficient analysis of small
patient samples (Bandura et al., 2009; Yao
et al., 2014). Multiparametric CyTOF
analysis of normal skin and in inflamma-
tory disease revealed a remarkable intra-
individual heterogeneity in homeostatic
DC populations and highly polarizing
impact of inflammatory diseases on
immune subsets (Alcántara-Hernández et al., 2017; Farrera et al., 2020).

Genome-based analysis has radically transformed our understanding of skin immunity. Spurred by the human genome project (Collins et al., 2003), the ability to sequence and compile whole-human genomes uncovered genetic susceptibility loci underlying a number of complex inflammatory skin diseases (Paternoster et al., 2011; Tsai et al., 2017). These studies provided key insights into the molecular and cellular drivers of complex multifactorial diseases. For instance, GWAS of Alopecia areata were instrumental in identifying the key innate and adaptive immune drivers of hair follicle destruction (Petukhova et al., 2010). Similarly, the IL-23 and NF-κB immune pathways were linked to psoriasis with GWAS (Nair et al., 2009).

Microarray technology and, more recently, RNA sequencing has provided a global picture of gene expression from skin tissue and purified immune cells (Li et al., 2014; Nirschl et al., 2017). Transcriptional analysis has also been instrumental in revealing the unique, universal, and synergistic cellular programs induced by inflammatory cytokines (Mehta et al., 2017; Swindell et al., 2018). Mechanistic studies using cell culture systems and animal models have defined the causal contributory factors identified by GWAS and transcriptional studies (Billi et al., 2020; Hawkes et al., 2017) paving the way for the development of targeted therapeutics.

The power of evaluating the gene expression of a single cell was harnessed by next-generation sequencing platforms to evaluate transcriptomes at cellular resolution (Tang et al., 2009). There has since been an explosion in the use of single-cell RNA sequencing (scRNAseq) by skin immunologists to study cellular heterogeneity (Shook et al., 2018), identify rare cell populations (Kobayashi et al., 2019b), and map the developmental (Popescu et al., 2019) and functional trajectories of distinct cell lineages (Tang et al., 2019). Comparing immune cells in psoriasis, atopic dermatitis, vitiligo, and bullous skin disease (Cheng et al., 2018; TK Hughes, unpublished data, 2019) has revealed heterogeneity not only in immune cells but also in the functionally responsive stromal cells that they engage. Although scRNAseq has been instrumental in mapping the cellular ecology of cutaneous immunity, just as the genomic techniques that came before, functional follow-up studies will be essential to determine causality and meaningful cellular interactions. Perhaps the most exciting application of scRNAseq is its use in rapid diagnosis, especially in diseases that lack a clear mechanism. Kim et al., (2020) recently used scRNAseq to effectively diagnose and treat a patient with drug-induced hypersensitivity syndrome, a disease with elusive pathophysiology.

Emerging technology and future promise

Many emerging technologies are melding methods to evaluate multiple modalities in the same sample. For instance, cellular indexing of transcriptomes and epigenomes by sequencing (STOECKIUS et al., 2017) combines antibody-based protein detection with scRNAseq, allowing for simultaneous evaluation of gene transcript and its protein product within a single cell. Similarly, concurrent assessment of epigenetic state, including chromatin accessibility, DNA and histone modifications and 3D chromatin structure, and the transcriptional landscape of a single cell, may provide a more nuanced understanding of regulatory genomic elements that underlie distinct cell states (JIA et al., 2018). One of the most exciting techniques on the horizon is spatial transcriptomics (MONCADA et al., 2020), a method that provides gene expression coupled with spatial distribution in tissue. Spatial transcriptomics will be particularly useful to evaluate microanatomical heterogeneity in disease, for instance, the tumour-stromal interface or the edge and bed of a nonhealing wound. Widely implementing these technologies will undoubtedly require tremendous computational power and the use of machine learning. An added challenge posed by these techniques is the integration of large datasets and dissemination for downstream functional validation. Nevertheless, these advances present a tantalizing toolbox with which cutaneous biologists can compose rich portraits of skin immune health and disease.

Data availability statement

No datasets were generated or analyzed during this study.

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CONFLICT OF INTEREST

The authors state no conflict of interest.

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AUTHOR CONTRIBUTIONS

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