

RESEARCH HIGHLIGHT



Spatiotemporal mapping of the leprosy granuloma landscape

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Leprosy is a chronic infectious disease of humans caused by *Mycobacterium leprae* (*M. leprae*) that is prevalent in some countries of Asia, Africa, and South America [1].

Q1–Q3

Leprosy has traditionally been classified into two major types, tuberculoid and lepromatous. Patients with tuberculoid leprosy (T-LEP) have limited disease with relatively few bacteria in the skin and nerves and can successfully control the infection. In contrast, lepromatous leprosy (L-LEP) patients have widespread disease and a large number of bacteria. Indeed, this disease spectrum is dynamic since patients may undergo a reversal reaction (RR) and generally upgrade, either spontaneously or during chemotherapy, from L-LEP to T-LEP [2].

The histological hallmark of leprosy is the formation of granulomas, organized structures formed by immune and nonimmune cells that serve to contain not only the infection but also the inflammatory response; otherwise, the result is deleterious to the host.

The structure of granulomas is distinct across the spectrum of leprosy. Granulomas found in T-LEP lesions are very well organized and characterized by fibrosis, with a core of mature macrophages and multinucleated giant cells, while lymphocytes are located at the periphery. Conversely, in L-LEP lesions, granulomas are disorganized and enriched in immature foamy macrophages with lymphocytes scattered throughout. Granulomas in lesions of RR patients are histologically similar to those in tuberculoid leprosy lesions [2].

Although several studies have characterized functional subsets of human T cells and macrophages and their cytokine secretion, the detailed structural organization of granulomas in different forms of leprosy and their cellular composition (including cell populations other than lymphocytes and macrophages) have yet to be defined [3]. Recent developments in single-cell RNA sequencing (scRNA-seq) are complementing and updating immunocytochemistry and flow cytometer methodologies, now providing us with the finest level of resolution for gene expression patterns in large sets of cells, unveiling much finer aspects and allowing their classification from human tissues.

In a study published in July in *Nature Immunology*, Feiyang Ma and colleagues [4] integrated data from scRNA-seq and spatial sequencing of five L-LEP and five RR biopsies from ten different patients to define the architecture of leprosy granulomas and the contribution of individual cell types to the antimycobacterial immune response.

The authors identified 12 diverse populations present in 21,318 cells from the 10 biopsy specimens, detecting an average of 741 genes and 3556 transcripts per cell. The annotated cell types

included T cells, B cells, plasma cells, myeloid cells, Langerhans cells, keratinocytes, fibroblasts, smooth muscle cells, endothelial cells, melanocytes, and exocrine gland cells. However, this cell distribution pattern consistently differed in the two clinical forms of leprosy: while the major cell types, including T cells, B cells, myeloid cells, keratinocytes, endothelial cells and fibroblasts, were found in both RR and L-LEP lesions, Langerhans cells were more abundant in RR lesions, and plasma cells were enriched in L-LEP lesions. Any of these populations were indeed heterogeneous, with 7 T cell and keratinocyte subclusters, 6 endothelial cell subclusters, and 5 myeloid and fibroblast subclusters (Table 1). Pseudotimed analysis using Monocle [5] additionally showed that, at least for the myeloid and keratinocyte populations, the cells of these subclusters represent a continuum from L-LEP- to RR-derived cells.

As expected, an *M. leprae* transcriptomic signature was found substantially in L-LEP lesions and very poorly represented in RR lesions. Conversely, antimicrobial genes were highly expressed in cell types from RR lesions, and the authors identified *IFNG* and *IL1B* as the upstream regulators of such genes using ingenuity pathway analysis. In addition, by superimposing data related to the cytokine signatures with the localization of the five most important subtypes of cells in the lesions, the authors assigned a specific cytokine imprint to each population. In the case of RR lesions, the imprint combined with the spatial distribution inside the lesion ensured success in walling the pathogen, which instead failed to be contained in the case of L-LEP lesions.

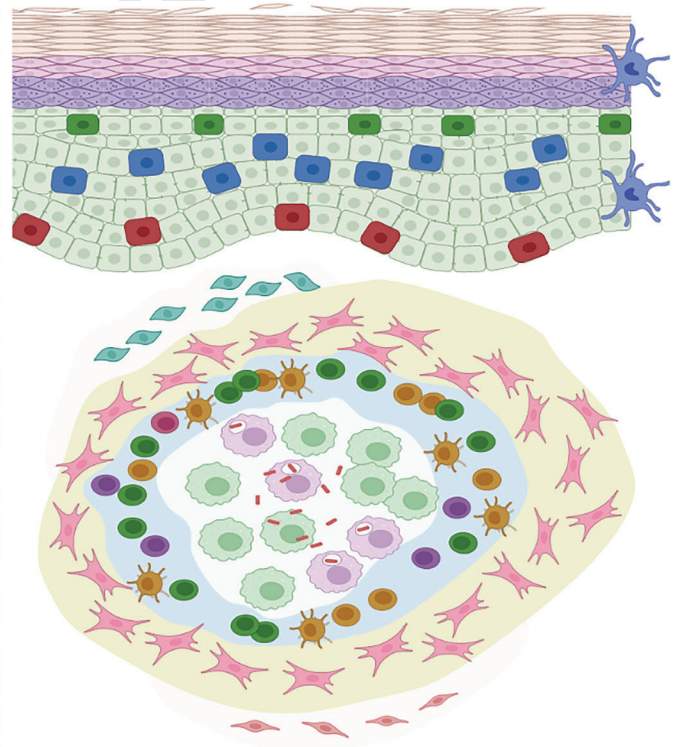
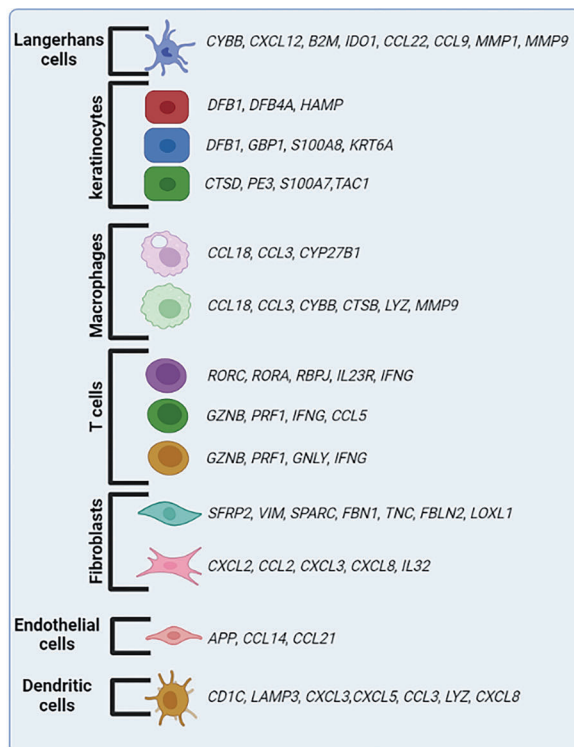
The scenario identified in the RR lesions shows several populations belonging to both the myeloid and lymphoid lineages, characterized by a specific antimicrobial signature. Four genes encoding antimicrobial molecules, *CYBB*, *CCL5*, *MMP2*, and *KLK5*, correspond to myeloid cells, T lymphocytes, fibroblasts, and granular keratinocytes, respectively. Other keratinocytes more frequent in RR than in L-LEP express antimicrobial genes such as *CXCL14*, *DEFB1*, and *TAC1*. The spatially distributed antimicrobial signature in the cells that make up the immunological ecosystem of RR lesions was further confirmed by the study of 66 genes associated with this type of signature.

Ma et al. [4] also used spatial sequencing to reconstruct the cytoarchitecture of the granuloma in detail, as well as the antimicrobial pathways that can develop within it. Specifically, the model of an organized granuloma consists of a core of transitional and M1-like macrophages. The populations of T lymphocytes that support the adaptive antimicrobial response are located more externally in the mantle zone, surrounded by dendritic cells and fibroblasts expressing genes of molecules with

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Table 1. Most significant cell populations, subsets, and signatures in RR and L-LEP lesions

Cell populations	Cell subsets	Signature	RR	L-LEP
T cells	TC0	Th17 (<i>RORC, RORA, RBPJ, IL23R, IFNG</i>)	+	±
	TC1	Cytotoxic (<i>CD8A, GZNB, PRF1, IFNG</i>)	+	±
	TC2	IFN I downstream (<i>IFI44L, MX1, IRF1,OAS3</i>), <i>CD8A, GZNB, PRF1, IFNG</i>	–	+
	TC3	Central Memory	+	+
	TC4	Naive	+	+
	TC5	Treg	+	+
	TC6	Antimicrobial (<i>GZNB, PRF1, GNLY, IFNG</i>)	+	–
Myeloid cells	ML0	<i>CD1C, LAMP3</i>	+	–
	ML1	IFN I downstream (<i>IFI44L, MX2,IFIT3,OAS3</i>)	–	+
	ML2	<i>TREM2, APOE</i>	–	+
	ML3	signature between ML2 and ML4	+	+
	ML4	M1-like macrophages (<i>TNF, IL1B</i>)	+	–
Keratinocytes	KC1	<i>KRT1, S100A14, LGALS3, LGALS7B</i>	–	+
	KC3	<i>FLG</i> granular layer, Antimicrobial Signature	+	–
	KC4	<i>KRT14/15</i> basal layer, Antimicrobial Signature	+	–
	KC5	<i>AREG, SNA12, ITGA6, ITGAV</i>	–	+
Fibroblasts	FB0	<i>SFRP2, VIM, SPARC, FBN1, TNC, FBLN2, LOXL1</i>	+	–
	FB1	<i>MGP</i>	–	+
	FB2	<i>CXCL2, CCL2, CXCL3, CXCL8, IL32</i>	+	–
	FB3	<i>COL11A1</i>	–	+
Endothelial cells	EC3	<i>MEOX2</i>	–	+
	EC4	<i>LYVE1</i>	+	–
	EC5	<i>HEY2</i>	+	–

**Fig. 1** Spatiotemporal architecture of granuloma in RR lesions, as defined by scRNA-seq and spatial sequencing. Shown is the positioning of the different cell types and their differentiation subsets in the context of RR granuloma, as derived by combined special and scRNA-seq. The gene signature of any cell type is indicated on the left

direct antimicrobial activity. Completing the structure are fibroblasts, endothelial cells, and various subpopulations of keratinocytes, all of which produce molecules with antimicrobial activity (Fig. 1). However, the role of the two different subsets of fibroblasts, those localized in the most superficial area of the dermis and those surrounding the granuloma's periphery, is different: the former produce a physical barrier by virtue of the deposition of the extracellular matrix, and the latter release antimicrobial molecules.

Thus, the spatial reconstruction of the granulomatous structure suggests an immunological environment consisting of different layers of cells that act in concert thanks to the regulation of the immune response driven by *IL1B* and *IFNG* and the ability to contain infections in RR lesions. Spatial sequencing of skin biopsies from one patient with T-LEP showed that the cell type composition in the T-LEP lesions was similar to that in the RR samples, further confirming the notion that RR represents a transition from L-LEP to T-LEP.

Conversely, Ma et al. found that L-LEP lesions consist mainly of macrophages expressing TREM2 (a molecule that identifies a subset of macrophages equipped with immunosuppressive activity [6]) that are programmed to transport and process lipids [7], culminating in the appearance of foamy cells that connote these lesions. Additionally, both the myeloid and cytotoxic T lymphocyte populations present in L-LEP lesions were enriched in the type I IFN gene signature and the abundance of plasma cell aggregates with local antibody production that contributed to the tissue damage present in these lesions.

There are two very important aspects of this study. First, and unexpectedly, nonimmune cells such as fibroblasts, keratinocytes and endothelial cells, which are not generally considered to contribute to the antimicrobial response, indeed expressed antimicrobial genes in RR granulomas. This suggests a yet unappreciated role of these cells in protective immune responses to microbial pathogens by their ability to produce a variety of antimicrobial molecules in response to IFN- γ and IL-1 β . Second, this study produced a detailed spatial and temporal model of the immune response against *M. leprae*. This response results in the activation of different cell subsets that organize themselves into a structure, the granuloma, that is capable of containing the infection. In other words, the concept of an immunological ecosystem emerges from this study, in which adjacent cell subsets perhaps influence each other spatially and temporally. Obviously, the lack of a suitable animal model does not allow analysis of the functional consequences of the differences in spatial localization and cellular interactions in L-LEP and RR or T-LEP lesions, and the question remains as to whether the relative spatial organizations of different cell types are important for function. Moreover, it would be important to understand whether the granuloma architectures described here are unique to leprosy or whether they extend to other granulomas, such as tuberculosis or the

comprehensive characterization of the lung immune cell landscape that has been recently documented in macaques [8]. Interestingly, several different myeloid subtypes (dendritic cells, type I IFN macrophages, TREM2 macrophages and transitional macrophages) described by Ma and colleagues in leprosy lesions correspond to similar cell populations found in the lung from tuberculosis macaques by a combination of scRNA-seq and CyTOF techniques [4, 8].

Nevertheless, the study by Ma et al. [4] provides a strong basis to understand why a single microbial pathogen is capable of inducing different clinical forms related to the quality of the immune response evoked against it.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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