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PROVA 1

1. In che cosa consiste la respirazione cellulare e dove avviene?

2. A cosa serve il programma Microsoft Excel?

nature immunology

LETTERS

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OPEN

Circadian clocks guide dendritic cells into skin lymphatics

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Migration of leukocytes from the skin to lymph nodes (LNs) via afferent lymphatic vessels (LVs) is pivotal for adaptive immune responses[1,2]. Circadian rhythms have emerged as important regulators of leukocyte trafficking to LNs via the blood[3,4]. Here, we demonstrate that dendritic cells (DCs) have a circadian migration pattern into LVs, which peaks during the rest phase in mice. This migration pattern is determined by rhythmic gradients in the expression of the chemokine CCL21 and of adhesion molecules in both mice and humans. Chronopharmacological targeting of the involved factors abrogates circadian migration of DCs. We identify cell-intrinsic circadian oscillations in skin lymphatic endothelial cells (LECs) and DCs that cogovern these rhythms, as their genetic disruption in either cell type ablates circadian trafficking. These observations indicate that circadian clocks control the infiltration of DCs into skin lymphatics, a process that is essential for many adaptive immune responses and relevant for vaccination and immunotherapies.

Steady-state migration of dermal DCs into afferent LVs is tightly regulated by a variety of promigratory factors, including the CCL21–CCR7 chemokine axis and the adhesion molecules LYVE-1, CD99 and JAM-A[1,2,5–12]. In the LN, ~24-h-long circadian rhythms influence the homing capacity and function of lymphocytes[4,13–16]; however, whether the draining of leukocytes from tissues occurs in a rhythmic manner is unknown.

To address this question, we collected mouse ear skin at different times of the day (that is, zeitgeber time 1 (ZT1; 1 h after light onset in a 12-h light/12-h dark environment; 'morning'), ZT7 ('day'), ZT13 ('evening') and ZT19 ('night')) and cultured the explants for 6 h in medium. We then quantified the location of tissue-endogenous CD11c$^+$ DCs inside LYVE-1$^+$ skin lymphatics by immunofluorescence imaging (Fig. 1a and Extended Data Fig. 1a,b). Infiltration of CD11c$^+$ cells into the LVs peaked at ZT7 (day) and troughed at ZT19 (night) (Fig. 1a and Supplementary Table 1). Additional quantification of the location of CD11c$^+$ DCs in the ear at steady state at ZT1, ZT7, ZT13 and ZT19 without ensuing culture confirmed a stronger intralymphatic presence of cells during the day than at night (Extended Data Fig. 1c). Explants that were collected at ZT7 and ZT19 and cultured for 24 h still exhibited higher CD11c$^+$ infiltration in LVs at ZT7 than at ZT19 (Fig. 1b and Extended Data Fig. 1b), indicating that night migration did not catch up to the day migration. The diurnal migration of CD11c$^+$ DCs into LVs was also detected after the topical application of fluorescein isothiocyanate (FITC), an inflammatory stimulus (Fig. 1c), indicating that the migration differences were maintained during inflammation. The amount of cells analyzed and the overall LV volume was similar at all time points (Extended Data Fig. 1d,e).

Circadian rhythms are defined by their persistence in the absence of external entraining factors, such as rhythmic light onset and offset. To investigate whether the oscillations in DC migration into LVs were circadian in nature, mice were housed in constant darkness. The migration differences of CD11c$^+$ DCs in LVs between the peak at ZT7 (day) and the trough at ZT19 (night) continued in conditions of constant darkness, demonstrating these oscillations to be bona fide circadian (Fig. 1d). The oscillations adjusted to a 12-h inverted light–dark cycle (Fig. 1d), indicating that they could be phase shifted and entrained by light, an additional feature of circadian rhythms. This indicated that CD11c$^+$ DC migration into skin LVs was driven by endogenous circadian rhythms and did not represent solely a response to a rhythmic day–night environment.

In situ whole-mount staining of ears identified that CD11c$^+$langerin$^-$ CD103$^-$ dermal conventional DCs (cDC2s) and CD11c$^+$langerin$^+$ Langerhans cells (LCs) preferentially migrated into LVs at ZT7 (day) compared to at ZT19 (night), while very few CD11c$^+$langerin$^-$CD103$^+$

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PROVA 2

1. Cosa è una mutazione "Driver"?

2. Con il termine "Firewall" si intende un software che ha specifica funzione. Quale?

Lymphatic endothelial cells of the lymph node

Sirpa Jalkanen[1,2] *and Marko Salmi*[1,2]✉

Abstract | The influx and efflux of cells and antigens to and from the draining lymph nodes largely take place through the subcapsular, cortical and medullary sinus systems. Recent analyses in mice and humans have revealed unexpected diversity in the lymphatic endothelial cells, which form the distinct regions of the sinuses. As a semipermeable barrier, the lymphatic endothelial cells regulate the sorting of lymph-borne antigens to the lymph node parenchyma and can themselves serve as antigen-presenting cells. The leukocytes entering the lymph node via the sinus system and the lymphocytes egressing from the parenchyma migrate through the lymphatic endothelial cell layer. The sinus lymphatic endothelial cells also orchestrate the organogenesis of lymph nodes, and they undergo bidirectional signalling with other sinus-resident cells, such as subcapsular sinus macrophages, to generate a unique lymphatic niche. In this Review, we consider the structural and functional basis of how the lymph node sinus system coordinates immune responses under physiological conditions, and in inflammation and cancer.

Fibroblastic reticular cells (FRCs). Specialized reticular fibroblasts located in the T cell areas of lymph nodes and other secondary lymphoid organs that produce collagen-rich reticular fibres and form stromal networks and conduits that are important for the trafficking of immune cells.

High endothelial venules Specialized venules that occur in secondary lymphoid organs, except the spleen. High endothelial venules allow continuous transmigration of lymphocytes as a consequence of the constitutive expression of adhesion molecules and chemokines on their luminal surface.

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Lymph nodes (LNs), and other secondary lymphatic tissues, generate highly specialized microenvironments for mounting effective immune responses[1,2]. In terms of adaptive immunity, the concentration of antigens, antigen-presenting cells and naive lymphocytes in the LNs enforces T cell and B cell activation and their differentiation into effector cells. Although the vast majority of LN cells (approximately 95%) are leukocytes, the different non-leukocytic stromal cell types, including blood vessel endothelial cells (BECs), lymphatic endothelial cells (LECs) and fibroblastic reticular cells (FRCs), are absolutely essential for the function of the organ[1,4]. Many lymphocyte types, such as naive cells and central memory cells, as well as other leukocyte types and their progenitors, enter the LN from the blood via high endothelial venules, which are lined by unique BECs that are specialized for supporting the multistep leukocyte extravasation cascade[5,6]. LNs also have an extensive lymphatic vasculature. Most leukocytes, including effector and memory lymphocytes, activated dendritic cells and monocytes, can enter the afferent lymphatics in peripheral tissues[7–10]. Antigens and other soluble molecules of the interstitial fluid also drain into the afferent lymph. The lymph-borne molecules and cells are then conveyed into the draining LN by unidirectional lymph flow. Within the LN, the afferent lymphatic vessels with circular cross-sectional profiles transform into several branched sinus systems that are lined by a layer of LECs. At the efferent side of the LN, lymphocytes use specialized lymphatic sinuses to enter the efferent lymphatic vasculature, which finally drains these cells back into the blood circulation. In addition to stromal BECs and LECs, multiple different subtypes of non-endothelial stromal cells, including FRCs, are found in LNs[11,12]. These mesenchyme-derived cells produce the collagen-based conduit system, give structural support to the organ and provide survival signals and routes for the directional migration of leukocytes between the different compartments of the LN. As LN blood vessels, FRCs and peripheral lymphatic vessels have been scrutinized in several excellent Reviews[1–6,8,12–15], we focus our discussion on the lymphatic vasculature within the LNs. Therefore, the numerous papers characterizing the immunology of the entire afferent arm of the lymphatic vasculature or the sinus leukocytes (without separating the LN LEC contribution) largely fall outside the scope of this Review.

New technologies have enabled the discovery of unanticipated heterogeneity of sinusoidal LECs. Because these cells form a physical barrier between the lymph-borne material and the LN parenchyma, where the responding naive lymphocytes reside, there has been increasing interest in defining the mechanisms by which antigens and different leukocyte types penetrate through the sinusoidal LECs. Moreover, many previously unappreciated functions, including LN organogenesis, antigen presentation and interactions with other sinus-resident cell types, such as subcapsular sinus macrophages, have also been recently ascribed to LN LECs. In reviewing these new insights into the architecture, phenotype and function of different LN LEC populations, we reinforce their key roles in supporting multiple aspects of immunosurveillance in both health and disease.

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PROVA 3

1. Che cosa sono le mutazioni?

2. Cosa si intende con il termine "Base di dati"?

ARTICLES
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Fibroblasts as a source of self-antigens for central immune tolerance

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Fibroblasts are one of the most common but also neglected types of stromal cells, the heterogeneity of which underlies the specific function of tissue microenvironments in development and regeneration. In the thymus, autoreactive T cells are thought to be negatively selected by reference to the self-antigens expressed in medullary epithelial cells, but the contribution of other stromal cells to tolerance induction has been poorly examined. In the present study, we report a PDGFRα$^+$ gp38$^+$ DPP4$^-$ thymic fibroblast subset that is required for T cell tolerance induction. The deletion of the lymphotoxin β-receptor in thymic fibroblasts caused an autoimmune phenotype with decreased expression of tissue-restricted and fibroblast-specific antigens, offering insight into the long-sought target of lymphotoxin signaling in the context of the regulation of autoimmunity. Thus, thymic medullary fibroblasts play an essential role in the establishment of central tolerance by producing a diverse array of self-antigens.

The thymic medulla provides a unique microenvironment in which virtually all the self-antigens are presented so that autoreactive T cells are eliminated from the T cell repertoire before reaching the periphery, thus establishing central T cell tolerance. Medullary thymic epithelial cells (mTECs) play the dominant role in this process by virtue of their promiscuous expression of tissue-restricted antigens (TRAs)[1,2]. In addition, a variety of cell types exist in the thymic medulla: hematopoietic cells such as lymphocytes, dendritic cells (DCs), and macrophages and terminally differentiated mTECs, including Hassall's corpuscles and thymic tuft cells[3,4]. Such a diversity of cell lineages in the thymus may contribute to the extensive coverage of the broad range of self-antigen expression by inducing genes specific to various cell types, rather than just certain specific genes restricted to a small number of tissues.

The thymic medulla contains non-TEC stromal cells of mesenchymal origin, although their immunological importance remains to be determined. Fibroblasts are neural crest-derived, mesenchymal stromal cells that also reside in the thymus[5–8]. Although it has been generally considered that fibroblasts are commonly distributed stromal cells without specific features, recent studies have attracted attention to the heterogeneity of fibroblasts in both the physiological and the pathological contexts[9]. Single-cell RNA-sequencing (RNA-seq) analyses revealed a vast heterogeneity of fibroblasts in mouse skin during the course of wound repair[10] and also identified disease-associated fibroblasts in rheumatoid arthritis and tissue fibrosis[11,12]. In secondary lymphoid organs, a specialized type of fibroblast termed 'fibroblastic reticular cells' (FRCs) constitutes a stromal network that allows lymphocyte trafficking and interaction in immune responses[13]. In the thymus, fibroblasts are predominantly localized to the medulla and capsule. Thymic medullary fibroblasts form a reticular architecture that resembles the stromal network in secondary lymphoid organs, whereas capsular fibroblasts form a monolayer that covers the surface of the thymus. It has been demonstrated that both medullary (mFbs) and capsular fibroblasts (capFbs) originate from the neural crest cells that surround the embryonic thymus primordium[6,7]. However, there have as yet been no molecular markers reported that allow these two fibroblast subsets to be distinguished, so whether mFbs contribute to T cell development remains to be elucidated.

In the present study, we identified cell-surface marker proteins of mFbs and determined their transcriptome profiles. We also found that the development of mature mFbs and the expression of mFb-specific genes are controlled by the lymphotoxin signal derived from developing thymocytes. The lymphotoxin signal has been reported to control T cell tolerance[14–16], but its underlying mechanism remained elusive. We demonstrated that the deletion of the lymphotoxin-β receptor (LTβR) in thymic fibroblasts caused a decreased expression of fibroblast-specific antigens and a breakdown of immune tolerance. These results indicate that mFbs contribute to the shaping of the T cell repertoire via the production of unique self-antigens, thus providing new insight into the mechanism underlying central tolerance induction.

Results

DPP4 expression segregates thymic fibroblast subsets. The cells expressing the fibroblast marker gp38 (podoplanin) are localized in the medulla as well as in the capsule of the mouse thymus (Fig. 1a). The gp38$^+$ mFbs form a reticular architecture in the proximity of mTECs expressing keratin 14 (K14) and Aire as well as CD4 or CD8 single-positive (SP) thymocytes but not with CD205-expressing cortical TECs (cTECs) (Fig. 1b). The gp38$^+$ thymic fibroblasts were found by flow cytometry analysis in the CD45$^-$Ter119$^-$EpCAM$^-$CD31$^-$PDGFRα$^+$ population (Fig. 1c).

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PROVA 4

1. Negli organismi pluricellulari quanti e quali tipi di mutazioni si riconoscono?

2. La seguente formula Excel "=SOMMA(B1:B10)" cosa calcola?

3. Leggere e tradurre

nature immunology

ARTICLES

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Interferon-λ modulates dendritic cells to facilitate T cell immunity during infection with influenza A virus

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Type III interferon (IFN-λ) is important for innate immune protection at mucosal surfaces and has therapeutic benefit against influenza A virus (IAV) infection. However, the mechanisms by which IFN-λ programs adaptive immune protection against IAV are undefined. Here we found that IFN-λ signaling in dendritic cell (DC) populations was critical for the development of protective IAV-specific CD8$^+$ T cell responses. Mice lacking the IFN-λ receptor (*Ifnlr1*$^{-/-}$) had blunted CD8$^+$ T cell responses relative to wild type and exhibited reduced survival after heterosubtypic IAV re-challenge. Analysis of DCs revealed IFN-λ signaling directed the migration and function of CD103$^+$ DCs for development of optimal antiviral CD8$^+$ T cell responses, and bioinformatic analyses identified IFN-λ regulation of a DC IL-10 immunoregulatory network. Thus, IFN-λ serves a critical role in bridging innate and adaptive immunity from lung mucosa to lymph nodes to program DCs to direct effective T cell immunity against IAV.

Type III interferons (IFN-λ) are members of the interferon (IFN) family and critical mediators of antiviral defenses[1–3]. IFN-λ family members have functions similar to type I IFN, and are structurally related to the IL-10 cytokine family, utilizing the IL-10R2 receptor subunit as a part of their heterodimeric receptor with IFN-λR1 chain[4]. Although IFN-λ has overlapping functions with type I IFNs, several studies have revealed a unique, non-redundant role for IFN-λ in mediating protection against disease and pathology in the liver and at mucosal and barrier surfaces[5–10].

Influenza A virus (IAV) is a respiratory pathogen that causes seasonal epidemics and also presents the potential for major pandemic outbreaks and global disease through continual emergence and re-emergence from avian reservoirs. The high incidence of IAV infection, coupled with low vaccine effectiveness during the 2017–2018 season, highlights the necessity to gain a better understanding of the processes that regulate the immune responses against infection, to guide efforts aimed at developing effective therapeutic and vaccine adjuvants to protect against infection[11].

As an innate immune and immune-regulatory cytokine, IFN-λ has the capacity to direct protective immunity against IAV infection. When administered intranasally following IAV challenge, IFN-λ restricts IAV infection without inducing excessive tissue damaging inflammation that is typically associated with type I IFN administration[12–15]. The protective and non-injurious effects of IFN-λ are attributed, in part, to the selective expression of IFN-λR1 compared to the broad expression of the type I IFN receptor (IFNAR), wherein IFN-λR1 is selectively expressed on epithelial cells, neutrophils, plasmacytoid dendritic cells and, potentially, natural killer (NK) cells that contribute to IAV immunity[12,13,16–20].

Although IFN-λ directs innate immune defenses that can restrict IAV infection, the contribution of IFN-λ signaling to adaptive immunity is less clear[21–23]. IFN-λ has been shown to enhance type I helper T cell responses in the lung, suggesting it has a role in regulating immune polarization during IAV infection[21,22]. Despite these observations, the direct function of IFN-λ on programming and maintenance of adaptive immune responses during IAV infection remains largely unknown. Here, we utilized murine infection models to investigate the role of IFN-λ (of which IFN-λ2 and IFN-λ3 are conserved between mice and humans) in adaptive immunity against IAV. We demonstrate that IFN-λ signaling is critical for the development of virus-specific CD8$^+$ T cells and generation of protective immunity that is able to provide cross-protection against heterosubtypic IAV re-challenge. We found that the blunted CD8$^+$ T cell response in IFN-λ receptor-deficient (*Ifnlr1*$^{-/-}$) mice is due to a defect in the activation, migration and function of dendritic cells (DCs), linked with aberrant expression of a DC-derived IL-10 immunoregulatory gene network. IFN-λ specifically programs migratory, antigen-presenting CD103$^+$ DCs to mediate CD8$^+$ T cell activation for broad protection against IAV infection and for heterosubtypic virus challenge that underscores contemporary IAV outbreaks in humans. Thus, IFN-λ, through the recruitment and function of CD103$^+$ DCs and regulation of IL-10 production, is an essential component of effective immunity against IAV infection and for cross-protection against heterosubtypic IAV emergence.

Results

IFN-λ signaling is required to generate protective memory T cell responses. To assess the role of IFN-λ signaling downstream of IFN-λR1 in controlling protection against secondary IAV infection we examined the response to heterosubtypic IAV challenge and determined the ability of *Ifnlr1*$^{-/-}$ mice to mount a protective homosubtypic and heterosubtypic (T cell-dependent) memory response against IAV infection. We interrogated both homo- and heterosubtypic re-challenge, as neutralizing antibody responses have been demonstrated to effectively prevent homosubtypic IAV infection, but heterosubtypic IAV infection is a common occurrence

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PROVA 5

1. Qual è la composizione biochimica della membrana cellulare?

2. Per scrivere una lettera quale software è più opportuno usare?

3. Leggere e tradurre

Organoids in immunological research

*Yotam E. Bar-Ephraim[1,3], Kai Kretzschmar[1,3] and Hans Clevers[1,2]**

Abstract | Much of our knowledge regarding the interactions between epithelial tissues and the immune system has been gathered from animal models and co-cultures with cell lines. However, unique features of human cells cannot be modelled in mice, and cell lines are often transformed or genetically immortalized. Organoid technology has emerged as a powerful tool to maintain epithelial cells in a near-native state. In this Review, we discuss how organoids are being used in immunological research to understand the role of epithelial cell–immune cell interactions in tissue development and homeostasis, as well as in diseases such as cancer.

Feeder cell
A cell line generated from isolated neonatal murine fibroblasts that have been selected for supporting optimal growth of epidermal keratinocytes in 2D culture.

Pluripotent stem cells
(PSCs). Cells with the potential to generate all embryonic tissues, such as embryonic stem cells.

Induced pluripotent stem cells
(iPSCs). Pluripotent cells generated in culture by the (over)expression of defined genetic factors in non-pluripotent cells such as somatic cells.

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Epithelial tissues line the boundaries of the mammalian body. For example, they are found in the skin, the gastrointestinal tract, the lungs and the thymus. Although haematopoietic cells are widely distributed throughout all tissues of the human body, the concentration of immune cells is highest in the epithelia (with the exception of the blood and lymphoid organs), where immune cells directly interact with epithelial cells[1–3]. These interactions have a role in both the maturation of immune cells (for example, in the thymus) and their activation (for example, upon wounding). Epithelial cells are the body's first point of contact with the pathogen-infested environment, and they are the first cells to respond to a pathogenic infection[4]. To safeguard homeostasis and provide a quick response to infection, epithelial cells collaborate closely with immune cells. However, epithelial cells belonging to different tissues react differently to pathogenic stimuli and, consequently, different immune cell subsets safeguard these tissues. Even within the intestine (FIG. 1a), differences in the composition of the epithelium and the microbiota along its length are mirrored by different subsets of tissue-resident immune cells[5]. The intricate interactions between the epithelium and specific components of the immune system not only are a prerequisite for preventing and containing pathogenic infections but also are important in preventing excessive immune activation, which can result in catastrophic tissue damage, as well as in the repair processes that follow tissue damage.

Many model systems for studying epithelial cells and their interactions with the immune system have been established over the years. Methods to culture epidermal stem cells on feeder cell layers have been used for more than 50 years[6]. Recent advances have enabled the long-term culture of adult stem cells of the intestine[7,8], liver[9–11], skin epidermis[12–14] and other epithelia[15–20] in a manner that closely mimics in vivo conditions and allows for disease modelling in vitro[21]. In the underlying approach, adult stem cells are embedded in extracellular matrix (ECM)-rich hydrogels and provided with a growth factor-defined culture medium[7,15,21]. This results in the proliferation of the epithelial stem cells into 3D cell clusters, coincident with the production of differentiated cell types. These near-native epithelial cell clusters are termed 'organoids' (BOX 1). Adult stem cell-enriched organoids can also be generated using tissue pieces composed of only epithelial cells[7] or both epithelial cells and stromal cells[8]. Organoids can also be generated from pluripotent stem cells (PSCs), either embryonic stem cells or induced pluripotent stem cells (iPSCs)[15,21]. Generally, organoids are defined as 3D cell clusters that are grown from (pluripotent or adult) stem cells and spontaneously organize into organ-like or tissue-like structures that are enriched for the cell types typically present in the tissue of origin (TABLE 1). Of relevance to this Review are organoids that represent organs in which epithelial cells have dominant functional roles, such as kidney[22], stomach[23] and intestine[24].

In contrast to feeder cell-based methods, organoid technology typically allows for long-term proliferation and differentiation of stem cells under defined (and per definition, therefore, serum-free) conditions[15,21]. These defined conditions ensure that organoid cultures are not influenced by batch-to-batch variations that can often occur in serum-based cultures. Furthermore, adult stem cell-derived organoids contain many types of (epithelial) differentiated cell that are typically present in the tissue of origin (FIG. 1b), and they are genetically stable and retain their epithelial phenotype over time[15,25]. Organoids are thus cellularly heterogeneous, in contrast to feeder cell-based cultures, which are often homogeneous. These features of organoids not only allow for a wide range of experimental applications, such as imaging, molecular analyses and gene editing[26], but also enable reductionist approaches to study the interaction of epithelial cells with immune cells. With the development of organoid models of cancer[27] and (other) immunogenic diseases such as inflammatory

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PROVA 6

1. Qual è il ruolo della membrana plasmatica

2. Cosa si intende per SPAM?

Thymic epithelial cell heterogeneity: TEC by TEC

Noam Kadouri, Shir Nevo, Yael Goldfarb and Jakub Abramson *

Abstract | The generation of a functional T cell repertoire in the thymus is mainly orchestrated by thymic epithelial cells (TECs), which provide developing T cells with cues for their navigation, proliferation, differentiation and survival. The TEC compartment has been segregated historically into two major populations of medullary TECs and cortical TECs, which differ in their anatomical localization, molecular characteristics and functional roles. However, recent studies have shown that TECs are highly heterogeneous and comprise multiple subpopulations with distinct molecular and functional characteristics, including tuft cell-like or corneocyte-like phenotypes. Here, we review the most recent advances in our understanding of TEC heterogeneity from a molecular, functional and developmental perspective. In particular, we highlight the key insights that were recently provided by single-cell genomic technologies and in vivo fate mapping and discuss them in the context of previously published data.

Thymic epithelial cells
(TECs). Specialized stromal cells found in the thymus that have the ability to present antigens on MHC class I and class II molecules to developing T cells (thymocytes). Their main known functions include the induction of T cell lineage commitment, positive selection of functional T cell clones and negative selection of self-reactive T cell clones.

Positive selection
A crucial checkpoint in αβ T cell development, exclusively facilitated by cortical thymic epithelial cells, that ensures only functionally competent T cell clones capable of recognizing peptide–MHC complexes with adequately high affinity continue in the developmental process. T cell clones that do not recognize peptide–MHC complexes with sufficient affinity die by neglect.

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The thymus is a specialized primary lymphoid organ whose main function is the production of immunologically competent T cells that can recognize and eliminate foreign antigens but that tolerate the body's own components[1]. T cell 'education' in the thymus is mainly orchestrated by thymic epithelial cells (TECs), which provide developing T cells with cues for their navigation, proliferation, differentiation and survival (BOX 1).

The TEC compartment has been divided historically into two major subsets, which differ in their anatomical localization and functional roles. Specifically, whereas the early checkpoints of the T cell developmental programme (T cell lineage commitment and positive selection) are orchestrated by cortical thymic epithelial cells (cTECs), later steps of T cell development, including negative selection of self-reactive thymocytes or their diversion into the FOXP3$^+$CD25$^+$ regulatory T (T_{reg}) cell lineage (agonist selection), are primarily mediated by medullary thymic epithelial cells (mTECs)[2,3].

In the past several years, however, it has become evident that cTECs and mTECs are not homogeneous compartments but rather are characterized by a high degree of internal heterogeneity (FIG. 1a). This increasing appreciation of TEC complexity, previously reviewed in REF.[4], is accompanied by growing confusion regarding the molecular and functional characterization of the individual TEC subsets. This may stem from the fact that the characterization and/or isolation of these subsets has been achieved using only a small number of surface markers, hence obscuring the composite picture of the TEC compartment.

Here, we review the most recent advances in our understanding of TEC heterogeneity from a molecular, functional and developmental perspective that have been provided by single-cell genomic technologies and in vivo fate mapping and discuss them in the context of previously published data. Specifically, we discuss thymic epithelial progenitor cells (TEPCs) and highlight some of the key open questions and controversies regarding their molecular characteristics and progenitor properties at different stages of thymic development. Furthermore, we review in detail our current understanding of cTEC and mTEC heterogeneity and development, with a particular focus on the diverse cell subsets that were recently found to compose the autoimmune regulator (AIRE)-negative mTEC compartment, including the mTEC I subset[5], CC-chemokine ligand 21 (CCL21)-expressing mTECs[6,7], podoplanin-expressing (PDPN$^+$) junctional thymic epithelial cells (jTECs)[8,9], corneocyte-like keratin type I cytoskeletal 10-positive (KRT10$^+$) mTECs (also known as post-AIRE mTECs or mTEC III)[5,10–12] and the newly identified DCLK1$^+$ thymic tuft cells (also known as mTEC IV)[5,13–15]. We hope that this Review not only provides the most up-to-date and comprehensive snapshot of the TEC atlas but also helps in integrating this information with previous knowledge and clarifying some of the outstanding questions from the past.

Thymic epithelial progenitor cells

Identification of the putative TEPCs has been one of the major challenges in the field. Despite marked progress in this direction in the past two decades, and convincing evidence for the existence of bipotent TEPCs that can

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PROVA 7

1. Qual è il ruolo del colesterolo nella membrana cellulare?

2. Qual è l'estensione tipica di un file scritto in EXCEL?

3. Leggere e tradurre

ARTICLES
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nature neuroscience

Circulating miR-181 is a prognostic biomarker for amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a relentless neurodegenerative disease of the human motor neuron system, where variability in progression rate limits clinical trial efficacy. Therefore, better prognostication will facilitate therapeutic progress. In this study, we investigated the potential of plasma cell-free microRNAs (miRNAs) as ALS prognostication biomarkers in 252 patients with detailed clinical phenotyping. First, we identified, in a longitudinal cohort, miRNAs whose plasma levels remain stable over the course of disease. Next, we showed that high levels of miR-181, a miRNA enriched in neurons, predicts a greater than two-fold risk of death in independent discovery and replication cohorts (126 and 122 patients, respectively). miR-181 performance is similar to neurofilament light chain (NfL), and when combined together, miR-181 + NfL establish a novel RNA-protein biomarker pair with superior prognostication capacity. Therefore, plasma miR-181 alone and a novel miRNA-protein biomarker approach, based on miR-181 + NfL, boost precision of patient stratification. miR-181-based ALS biomarkers encourage additional validation and might enhance the power of clinical trials.

ALS is a devastating neurodegenerative disorder of the motor neuron system for which no effective disease-modifying treatment exists. ALS is characterized by substantial variability in progression rates[1,2], posing a considerable challenge for patient stratification in clinical trials. Thus, reliable predictors of disease progression would be invaluable for ALS patient stratification before enrollment in clinical trials. Ideal biomarkers should remain stable during the course of disease, be detectable in accessible tissues and also be easily measurable. To date, intensive research has identified only a few potential blood-based ALS biomarkers[3–5], including cell-free neurofilaments[6–8] and pro-inflammatory cytokines[9–11]. NfL was the first blood biomarker to aid in predicting ALS progression rate, but additional markers are needed to improve stratification and allow for more effective trials.

miRNAs are endogenous non-coding RNAs that are essential for motor neuron survival and have been shown to be globally downregulated in postmortem ALS motor neurons[12–14]. Although circulating miRNA profiles have been previously characterized in ALS[15–18], the potential of miRNA biomarkers for ALS prognosis and as readout of disease progression has not been fully explored.

Here we take a hypothesis-free approach by applying next-generation RNA sequencing (RNA-seq) to comprehensively study plasma miRNAs in a large cohort of 252 patients with ALS. We focused our attention on the miR-181 family, in which miRNAs are expressed from two homolog polycistronic genes: *mir-181a-1/b-1* (human chromosome 9) and *mir-181a-2/b-2* (human chromosome 1). The mature miR-181 species are functionally identical in silencing a single set of mRNA targets. We reveal that miR-181 levels predict disease progression in large discovery and replication cohorts and demonstrate the effectiveness of combining miR-181 with established NfL as a prognostic biomarker combination for ALS.

Results

Longitudinal study of circulating miRNAs in ALS. In this study, we explored blood-borne miRNAs as potential prognostic biomarkers for ALS. We used unbiased next-generation sequencing to investigate, without an a priori bias, the comprehensive landscape of plasma miRNAs in 252 patients with ALS, for whom documented clinical and demographic information is available (Table 1).

A crucial feature for a prognostic biomarker is its stability across the disease course. We, therefore, initially investigated a longitudinal sample cohort of 22 patients (clinical data in Table 2), with four longitudinal blood samples taken (t_1–t_4) during the course of 30 months (2.5 years). Eighty-eight samples (corresponding to the first cohort of 22 patients) were prepared from total plasma RNA, as previously described[20], and profiled by RNA-seq for miRNA levels. Linear miRNA quantification was achieved via 12-nucleotide unique molecular identifiers (UMIs). miRNAs with ≥50 UMIs in at least 60% of the samples (>53 of 88 samples) were considered above noise level. Thus, of 2,008 miRNAs aligned to the human genome (GRCh37/hg19), 187 passed our set expression threshold (Supplementary Table: Source Data Fig. 1). Next, to reduce noisy miRNAs, we excluded from further analysis 58 miRNAs with high variability (t_4/t_1 standard error ratio ≥ 0.2; Fig. 1a, *y* axis). For example, miR-181a-5p variability across individual patients is limited relative to that of miR-1-3p (*F*-test for variance = 20.9, $P<0.0001$;

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PROVA 8

1. In che modo gli organismi producono energia in assenza di ossigeno?

2. Se si vuole realizzare una presentazione quale programma è opportuno usare?

3. Leggere e tradurre

ARTICLES
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nature neuroscience

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Human ALS/FTD brain organoid slice cultures display distinct early astrocyte and targetable neuronal pathology

Kornélia Szebényi[1], Léa M. D. Wenger[1,6], Yu Sun[2,3,6], Alexander W. E. Dunn[4], Colleen A. Limegrover[1], George M. Gibbons[1], Elena Conci[1], Ole Paulsen[4], Susanna B. Mierau[4], Gabriel Balmus[2,3]✉ and András Lakatos[1,5]✉

Amyotrophic lateral sclerosis overlapping with frontotemporal dementia (ALS/FTD) is a fatal and currently untreatable disease characterized by rapid cognitive decline and paralysis. Elucidating initial cellular pathologies is central to therapeutic target development, but obtaining samples from presymptomatic patients is not feasible. Here, we report the development of a cerebral organoid slice model derived from human induced pluripotent stem cells (iPSCs) that recapitulates mature cortical architecture and displays early molecular pathology of *C9ORF72* ALS/FTD. Using a combination of single-cell RNA sequencing and biological assays, we reveal distinct transcriptional, proteostasis and DNA repair disturbances in astroglia and neurons. We show that astroglia display increased levels of the autophagy signaling protein P62 and that deep layer neurons accumulate dipeptide repeat protein poly(GA), DNA damage and undergo nuclear pyknosis that could be pharmacologically rescued by GSK2606414. Thus, patient-specific iPSC-derived cortical organoid slice cultures are a reproducible translational platform to investigate preclinical ALS/FTD mechanisms as well as novel therapeutic approaches.

Cerebral organoids represent a promising tool for understanding human brain physiology and disease processes[1]. Their resemblance to the human cerebral cortex—in terms of their three-dimensional (3D) architecture, cell-type diversity and cell–cell interactions[2,3]—provides major advantages over other stem-cell-derived culture and mouse models. Organoids provide a suitable biological platform for assessing dynamic sequences of human-specific cellular events, especially those relevant to neurodegenerative disease research, in which many cell types are now implicated in pathogenesis. However, the timing and cell-specific characteristics of molecular disturbances remain unclear[4]. Such investigations could help treatment strategies aiming to prevent pathological triggers. Since the experimental use of samples from presymptomatic human patients is not feasible, cerebral organoid technologies raise the possibility of capturing the initiating cell-type-specific pathologies of neurodegenerative diseases.

Despite recent technical improvements in organoid culture methods[5–7], there are a number of challenges remaining for neurodegenerative disease modeling. Brain organoids have been grown from cells derived from patients with Parkinson's disease and from patients with Alzheimer's disease for 30 or 84 days in vitro (DIV), respectively[8,9]. This has been a considerable step forward in disease model development; however, the relatively short longevity and variable cortical-cell-type composition[10,11] may limit the fidelity for observing a broad spectrum of pathology. In addition, nutrient and oxygen supply[12] is a particular problem in non-vascularized organoids, restricting growth and viability and therefore potentially the final steps of cell differentiation and maturation. Recent reports suggest that slicing brain organoids derived from embryonic stem cells and/or cultivating them on fenestrated membranes improve viability and potentially cell composition[5,6]. Similar advances are a prerequisite for patient-specific iPSC-derived organoids for precise pathomechanistic discoveries. This is particularly warranted for ALS/FTD, an untreatable neurodegenerative disease with rapid cognitive decline and paralysis.

Here, we report the development of a long-term human cortical organoid (CO) model that closely recapitulates early molecular pathology of ALS/FTD. COs were grown up to 240 DIV at the air–liquid interface (ALI-COs) from iPSCs derived from patients with ALS/FTD, harboring the *C9ORF72* hexanucleotide repeat expansion mutation (C9 ALI-COs). This mutation is useful for ALS/FTD modeling as it gives rise to a wide range of pathologies in both sporadic and inherited disease forms[13]. Using 587 ALI-CO slices cultured from human iPSC (hiPSC)-derived organoids, we show that ALI-COs develop consistent microarchitecture and mature cortical-circuit-forming disease-relevant phenotypes. Furthermore, we found that C9 ALI-COs, although lacking microglia and vasculature, exhibit astroglia- and neuron-specific disturbances. These include distinct transcriptional and proteostasis disturbances in astroglia and neurons, including early accumulation of the autophagy signaling protein P62 and the toxic dipeptide repeat protein (DPR) poly(GA), respectively. Deep layer neurons (DLNs) display DNA damage and cell death that we pharmacologically rescued by improving proteostasis. Our results demonstrate that hiPSC-derived ALI-COs provide a reproducible platform with the necessary longevity and maturity for investigating ALS/FTD, thereby revealing

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PROVA 9

1. Come avviene la duplicazione della cellula batterica?

2. In Excel cosa è una "funzione"?

nature neuroscience

RESOURCE

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Neuroinflammatory astrocyte subtypes in the mouse brain

Philip Hasel[1]✉, Indigo V. L. Rose[1], Jessica S. Sadick[1], Rachel D. Kim[1] and Shane A. Liddelow[1,2,3]✉

Astrocytes undergo an inflammatory transition after infections, acute injuries and chronic neurodegenerative diseases. How this transition is affected by time and sex, its heterogeneity at the single-cell level and how sub-states are spatially distributed in the brain remains unclear. In this study, we investigated transcriptome changes of mouse cortical astrocytes after an acute inflammatory stimulus using the bacterial cell wall endotoxin lipopolysaccharide. We identified fast transcriptomic changes in astrocytes occurring within hours that drastically change over time. By sequencing ~80,000 astrocytes at single-cell resolution, we show that inflammation causes a widespread response with subtypes of astrocytes undergoing distinct inflammatory transitions with defined transcriptomic profiles. We also attribute key sub-states of inflammation-induced reactive astrocytes to specific brain regions using spatial transcriptomics and in situ hybridization. Together, our datasets provide a powerful resource for profiling astrocyte heterogeneity and will be useful for understanding the biological importance of regionally constrained reactive astrocyte sub-states.

Astrocytes perform crucial homeostatic functions such as neurotransmitter uptake and recycling of metabolites to support active neurons[1,2] and form an integral part of the blood–brain barrier protecting the brain from toxic metabolites and peripheral insults[3]. They also undergo inflammatory transitions after acute insults like stroke[4] and spinal cord injury[5], as well as in chronic diseases like Alzheimer's disease, and during normal ageing[6–8]. This 'reactive' response depends on the initiating insult[3,6]. For example, systemic inflammation causes a neurotoxic reactive subtype induced by microglial cytokine release[6]. However, whether this inflammatory insult induces a single homogeneous population or other multiple heterogeneous astrocyte subsets is not known. Recent work has shown that astrocytes have distinct gene expression profiles depending on brain region or cortical layer[9–14]. Similarly, the astrocytic transcriptome can be affected by neuronal contact or neuronal activity[13]. We decided to investigate whether astrocyte heterogeneity is maintained during responses to inflammation and if additional layers of heterogeneity are present.

We induced systemic inflammation in mice using the endotoxin lipopolysaccharide (LPS) and used a multimodal approach consisting of in vitro and in vivo bulk RNA-sequencing (RNA-seq), single-cell RNA-seq (scRNA-seq), spatial transcriptomics, NanoString gene profiling and dataset integration to profile the astrocytic response to an inflammatory insult. These data provide valuable insights to the temporal, spatial and single-cell responses of astrocytes to inflammation and give insights into the evolution of heterogeneous responses of astrocyte to a single stimulus while simultaneously highlighting some degree of homogeneity across astrocyte subtypes.

Results

Astrocytes acquire distinct inflammatory states over time. To investigate astrocytic time-dependent transcriptome changes to inflammation, we injected postnatal day 30–35 (P30–35) *Aldh1l1*^{eGFP} reporter mice intraperitoneally with LPS or endotoxin-free saline as control. Although LPS does not cross the blood–brain barrier extensively[16], it mounts an inflammatory response in microglia required to induce astrocyte reactivity[6]. Three, 24 and 72 h after LPS injection, cortical astrocytes were fluorescence-activated cell sorting (FACS) purified, and total RNA was extracted and processed for bulk RNA-seq (Fig. 1a and Extended Data Fig. 1a). We found extensive changes in astrocytic gene expression at 3 h (383 Up, 128 Down), 24 h (442 Up, 158 Down) and 72 h (126 Up, 4 Down) (Fig. 1b,c and Supplementary Table 1). The response at 24 h was similar to that published previously[4], with common astrocyte reactivity markers *Gfap* and *Serpina3n* upregulated at all time points (Fig. 1e, Supplementary Table 2 and Supplementary Table 3). We found, however, that changes in gene expression were mostly unique to individual time points (Fig. 1d and Supplementary Table 2), with only 4% of 743 altered genes induced across all time points. For example, interferon stimulated genes (ISGs) such as *Ifgp1*, *Cxcl10* and *Gbp2* are expressed highest at 3 h (Fig. 1e,f and Supplementary Table 2). At 24 h, genes involved in angiogenesis and blood pressure like *Fbln5*, *Agt* and genes involved in extracellular matrix remodeling including *Timp1* are upregulated (Fig. 1e,f and Extended Data Fig. 2). In particular, *Fbln5* is interesting given its involvement in cell adhesion and vascular constriction during inflammation[17]. To contextualize these changes in terms of (probable) function, we used the Gene Ontology (GO) tool Metascape[18] (Extended Data Fig. 2 and Supplementary Table 3). We found unique and shared GO terms across all time points (Extended Data Fig. 2 and Supplementary Table 3). Examples of shared GO terms include 'adaptive immune response', 'NF-kappa B signaling pathway' and 'response to type I interferons'. We also found GO terms specific for each time point, which, at 3 h, included 'positive regulation of interleukin-12 production', 'interleukin-1 mediated signaling pathway' and 'interferon-gamma production'. At 24 h, these included 'regulation of coagulation', 'tissue remodeling' and 'antigen processing

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PROVA estratta

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PROVA 10

1. Quali sono le cellule di un organismo in grado di espletare la fagocitosi?

2. Cos'è il pacchetto Office?

3. Leggere e tradurre

nature microbiology

ANALYSIS

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Precise quantification of bacterial strains after fecal microbiota transplantation delineates long-term engraftment and explains outcomes

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Fecal microbiota transplantation (FMT) has been successfully applied to treat recurrent *Clostridium difficile* infection in humans, but a precise method to measure which bacterial strains stably engraft in recipients and evaluate their association with clinical outcomes is lacking. We assembled a collection of >1,000 different bacterial strains that were cultured from the fecal samples of 22 FMT donors and recipients. Using our strain collection combined with metagenomic sequencing data from the same samples, we developed a statistical approach named Strainer for the detection and tracking of bacterial strains from metagenomic sequencing data. We applied Strainer to evaluate a cohort of 13 FMT longitudinal clinical interventions and detected stable engraftment of 71% of donor microbiota strains in recipients up to 5 years post-FMT. We found that 80% of recipient gut bacterial strains pre-FMT were eliminated by FMT and that post-FMT the strains present persisted up to 5 years later, together with environmentally acquired strains. Quantification of donor bacterial strain engraftment in recipients independently explained (precision 100%, recall 95%) the clinical outcomes (relapse or success) after initial and repeat FMT. We report a compendium of bacterial species and strains that consistently engraft in recipients over time that could be used in defined live biotherapeutic products as an alternative to FMT. Our analytical framework and Strainer can be applied to systematically evaluate either FMT or defined live bacterial therapeutic studies by quantification of strain engraftment in recipients.

Fecal microbiota transplantation (FMT)[1] has been widely used to treat recurrent *Clostridium difficile* infection (CDI) since its superiority to vancomycin was demonstrated[2,3]. Several studies have shown that the recipient's gut microbiota post-FMT resembles that of a healthy donor, suggesting gut microbiota restoration[4,5]. However, these studies have failed to capture the most basic principle of commensal Koch's postulates[6,7], which is the identification of discrete bacterial strains from the donor that are isolated as a pure culture from the cured recipient only post-FMT and not before. Eight years and tens of thousands of successful FMTs[8] after the first successful clinical trial, there are basic but unanswered questions about how FMT alters the recipient human gut microbiota. Which strains in the FMT donor stool do or do not engraft is not clear. Which strains that colonized before FMT in recipients persist after the transplant and whether engrafting and persisting strains are durable members of the resulting microbiota is unresolved. The proportion of the recipient microbiota from the donor, recipient or environment has not been clarified. Whether any of these properties of strain engraftment and persistence predict relapse post-FMT has also not been reported.

We know that the functional impact of the gut microbiota is at the level of strains[9–13]. Therefore, quantification of gut microbiota at this resolution is essential for understanding the therapeutic potential of FMT and its impact on the health and disease of the host. Recently, several US Food and Drug Administration (FDA) advisories[14,15] related to severe adverse events from FMT have increased safety concerns around undefined FMT, which uses the entire stool. Strain-level resolution and tracking of the transmission of culturable discrete strains during FMT could provide a route towards the application of a therapeutically defined cocktail[16] of microbes as a safer and scalable alternative to FMT.

Achieving strain-level resolution in metagenomics has been challenging because the human gut microbiota consists of numerous bacterial strains in every species[17], most of which have not been isolated or even detected using sequencing. As a result, previous microbiome analyses[18–20] have focused on a lower level of resolution because finding delineating features of discrete bacterial strains, a necessary step for FMT strain tracking, is a challenge. While informative, metagenomics-only approaches[4,21,22] require very deep sequencing to track strains using single-nucleotide polymorphisms (SNPs) in marker genes, do not model the microbiota as a defined set of discrete strains and mainly provide non-quantifiable inferences related to sharing of metagenome-assembled bacterial contigs or SNPs across FMT samples. The linking of bacterial genetic variation to a discrete unit, that is, a cultured bacterial isolate, is essential if we are to understand the transmission of strains during FMT and fulfil commensal Koch's postulates[6,7].

To enable precise strain tracking, we developed a high-throughput hybrid approach, where we first cultured and also sequenced the genomes of strains from FMT donors and recipients using published methods[23–25] and then tracked these strains across metagenomic samples using the statistical method presented in this article, which we named Strainer.

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PROVA 11

1. Che cosa accadrebbe alla cellula se fosse priva di apparato del Golgi?

2. Se in Windows apriamo il programma "Paint" cosa abbiamo intenzione di fare?

3. Leggere e tradurre

ARTICLES
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nature microbiology

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Antibody responses to SARS-CoV-2 vaccines in 45,965 adults from the general population of the United Kingdom

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We report that in a cohort of 45,965 adults, who were receiving either the ChAdOx1 or the BNT162b2 SARS-CoV-2 vaccines, in those who had no prior infection with SARS-CoV-2, seroconversion rates and quantitative antibody levels after a single dose were lower in older individuals, especially in those aged >60 years. Two vaccine doses achieved high responses across all ages. Antibody levels increased more slowly and to lower levels with a single dose of ChAdOx1 compared with a single dose of BNT162b2, but waned following a single dose of BNT162b2 in older individuals. In descriptive latent class models, we identified four responder subgroups, including a 'low responder' group that more commonly consisted of people aged >75 years, males and individuals with long-term health conditions. Given our findings, we propose that available vaccines should be prioritized for those not previously infected and that second doses should be prioritized for individuals aged >60 years. Further data are needed to better understand the extent to which quantitative antibody responses are associated with vaccine-mediated protection.

Multiple vaccines have been developed that offer protection against COVID-19 by generating immune responses against the spike antigen of SARS-CoV-2. On 8 December 2020, the United Kingdom (UK) started its national vaccination programme with the Pfizer–BioNTech BNT162b2 vaccine[1], followed by the approval of the Oxford–AstraZeneca ChAdOx1 nCoV-19 vaccine, first used outside a clinical trial on 4 January 2021 (ref. [2]). Both vaccines have been widely used in the UK.

Vaccines were initially administered to priority groups, including care home residents, people >80 years old, healthcare workers and those clinically vulnerable (≥16 years), and then offered to the rest of the adult (≥18 years) population in decreasing age order[3]. To maximize initial coverage, in early January 2021, the dosing interval was extended to 12 weeks for all vaccines, regardless of the licensed dosing schedule. Up until 6 April 2021, 31.7 million people (60.2% of the population aged ≥18 years) have been given a first dose, and 5.7 million people (10.8%) have received two vaccine doses (https://coronavirus.data.gov.uk/details/vaccinations).

The efficacy of the ChAdOx1 and BNT162b2 vaccines against symptomatic laboratory-confirmed SARS-CoV-2 infection has been reported in large randomized controlled clinical trials as 52% (95% confidence interval (CI) = 30–86%) after the first dose and 95% (95% CI = 90–98%) after the second dose of BNT162b2 (ref. [4]), and 70% (95% CI = 55–81%) after the second dose of ChAdOx1 (ref. [5]). Several studies have examined the immunogenicity of vaccines in healthcare workers, who were typically the earliest groups to be vaccinated. A study of 3,610 healthcare workers found that 99.5% and 97.1% seroconverted after a single dose of BNT162b2 or ChAdOx1, respectively, and that higher quantitative immunoglobulin G (IgG) levels were achieved in previously infected individuals[6]. Other studies have also found that single-dose BNT162b2 elicited higher antibody levels in previously seropositive individuals, levels that were comparable to those after two doses of vaccines in seronegative individuals[7–9]. Outside trials, there are limited data on post-vaccine antibody responses in other groups, especially older adults who were underrepresented in the ChAdOx1 trial[5]. A study of 185 individuals aged >70 years showed high seropositivity after one or two BNT162b2 doses[10]. Another study, of 100 individuals aged 80–100 years, showed almost universal high antibody responses 3 weeks after a single dose of BNT162b2, with spike-specific cellular responses in 63% of participants[11]. However, the representativeness of these small cohorts is unclear.

Real-world data provide information on populations who may not participate in clinical trials and can be used to assess the efficacy

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PROVA 12

1. Com'è costituita l'atmosfera all'interno di incubatori per colture cellulari?

2. In informatica cosa si intende per bit?

nature neuroscience

ARTICLES

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Single-cell profiling of CNS border compartment leukocytes reveals that B cells and their progenitors reside in non-diseased meninges

David Schafflick[1,8], Jolien Wolbert[1,8], Michael Heming[1,8], Christian Thomas[2], Maike Hartlehnert[1], Anna-Lena Börsch[1], Alessio Ricci[3], Sandra Martín-Salamanca[4], Xiaolin Li[1], I-Na Lu[1], Mathias Pawlak[5], Jens Minnerup[1], Jan-Kolja Strecker[1], Thomas Seidenbecher[6], Sven G. Meuth[1], Andres Hidalgo[4], Arthur Liesz[3,7], Heinz Wiendl[1] and Gerd Meyer zu Horste[1]✉

The CNS is ensheathed by the meninges and cerebrospinal fluid, and recent findings suggest that these CNS-associated border tissues have complex immunological functions. Unlike myeloid lineage cells, lymphocytes in border compartments have yet to be thoroughly characterized. Based on single-cell transcriptomics, we here identified a highly location-specific composition and expression profile of tissue-resident leukocytes in CNS parenchyma, pia-enriched subdural meninges, dura mater, choroid plexus and cerebrospinal fluid. The dura layer of the meninges contained a large population of B cells under homeostatic conditions in mice and rats. Murine dura B cells exhibited slow turnover and long-term tissue residency, and they matured in experimental neuroinflammation. The dura also contained B lineage progenitors at the pro-B cell stage typically not found outside of bone marrow, without direct influx from the periphery or the skull bone marrow. This identified the dura as an unexpected site of B cell residence and potentially of development in both homeostasis and neuroinflammation.

The brain and spinal cord are ensheathed by cerebrospinal fluid (CSF) and fibrous membranes termed meninges that form three layers: the outer dura layer adjoining the skull, the middle arachnoid layer forming a meshwork filled with CSF and the inner pia layer covering the CNS surface[1]. Together with the choroid plexus (CP) that produces the CSF, these meninges have long been known to provide mechanical protection and trophic support to the CNS.

The (re-)discovery of lymphatic vessels in the dura[2,3] recently ignited interest in immune-related functions of such CNS-associated border compartments. In fact, meningeal lymphatics contribute to neuroinflammation[4]. However, leukocytes in these border compartments have mostly been investigated using biased approaches[5,6]. More recent studies provided an unbiased characterization, but analyzed leukocytes in all meningeal layers combined[7], focused on myeloid lineages[8,9] or did not restrict their analysis to tissue-resident leukocytes (TRLs)[5]. A deep characterization of lymphocytes residing in CNS-associated border compartments is thus currently unavailable.

We here systematically analyzed the composition of TRLs in border compartments of healthy rodents and identified unique cellular compositions. Only the dura contained a large proportion of homeostatic B cells (Bcs) located in lymphatic vessels and in the dura tissue that were also present in humans. Murine dura Bcs were long-term tissue resident and showed signs of local maturation in experimental neuroinflammation. Surprisingly, healthy rodent dura also contained Bc progenitors at the pro- and pre-Bc stage that were also long-term tissue resident. We thus identify the dura as a site of Bc residence and development.

Results

Excluding intravascular leukocytes by intravenous labeling. We aimed to better understand leukocytes residing in CNS-associated border compartments, namely: CNS parenchyma, pia-enriched subdural meninges (pia and arachnoid; SDM), dura, CP and CSF (Fig. 1a). Intracardiac perfusion alone was previously shown to not reliably remove blood leukocytes from tissue, thus adding substantial bias[10]. We tested whether the same was true for CNS border compartments and intravenously injected fluorophore-labeled CD45 antibody (CD45iv) before perfusion and gated on CD45iv$^-$CD45$^+$ cells, representing TRLs (Fig. 1b). After perfusion, CD45iv$^+$CD45$^+$ blood-derived non-TRLs still remained detectable in CNS parenchyma (hereafter, "CNS" refers to CNS parenchyma; 0.53% ± 0.41% s.d.), in SDM (2.82% ± 0.91% s.d.), in CP (3.77% ± 4.18% s.d.) and especially in dura (17.77% ± 11.03% s.d.) (Extended Data Fig. 1a). Intravascular labeling is thus indispensable for defining tissue-resident cells.

Unique leukocyte composition in each CNS border tissue. To better characterize CNS border-resident leukocytes, we performed single-cell RNA sequencing (scRNA-seq) of TRLs sorted from these compartments of wild-type (WT) rats in comparison with blood and CSF (Methods). Rats were chosen because they provided

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PROVA 13

1. Perché l'mRNA può formare ibridi solo con il tratto codificante del DNA?

2. Cosa indica il termine "Open Source"?

3. Leggere e tradurre

ARTICLES
https://doi.org/10.1038/s41564-021-00912-0
nature microbiology
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Gut microbiome alterations in high-fat-diet-fed mice are associated with antibiotic tolerance

Yuan Liu[1,2,3]✉, Kangni Yang[1], Yuqian Jia[1], Jingru Shi[1], Ziwen Tong[1], Dan Fang[1], Bingqing Yang[1], Chengrui Su[1], Ruichao Li[1,2,3], Xia Xiao[1,2] and Zhiqiang Wang[1,2]✉

Antibiotic tolerance, the ability of a typically susceptible microorganism to survive extended periods of exposure to antibiotics, has a critical role in chronic and recurrent bacterial infections, and facilitates the evolution of antibiotic resistance. However, the physiological factors that contribute to the development of antibiotic tolerance, particularly in vivo, are not fully known. Despite the fact that a high-fat diet (HFD) is implicated in several human diseases, the relationship between HFD and antibiotic efficacy is still poorly understood. Here, we evaluated the efficacy of multiple clinically relevant bactericidal antibiotics in HFD-fed mice infected with methicillin-resistant *Staphylococcus aureus* (MRSA) or *Escherichia coli*. We found that HFD-fed mice had higher bacterial burdens and these bacteria displayed lower susceptibility to bactericidal antibiotic treatment compared with mice that were fed a standard diet, while microbiota-depleted standard-diet- or HFD-fed mice showed similar susceptibility. Faecal microbiota transplantation from HFD-fed mice impaired antibiotic activity in mice fed a standard diet, indicating that alteration of the gut microbiota and related metabolites in HFD-fed mice may account for the decreased antibiotic activity. 16S rRNA sequencing and metabolomics analysis of faecal samples revealed decreased microbial diversity and differential metabolite profiles in HFD-fed mice. Notably, the tryptophan metabolite indole-3-acetic acid (IAA) was significantly decreased in HFD-fed mice. Further in vitro studies showed that IAA supplementation inhibited the formation of bacterial persisters and promoted the elimination of persisters in combination with antibiotic treatment, potentially through the activation of bacterial metabolic pathways. In vivo, the combination of IAA and ciprofloxacin increased the survival rate of HFD-fed mice infected with MRSA persisters. Overall, our data reveal that a HFD has an antagonistic effect on antibiotic treatment in a mouse model, and this is associated with the alteration of the gut microbiota and IAA production.

Antibiotics have had an unprecedented role in the control of bacterial infections[1]. However, the emergence of antibiotic-refractory pathogens severely decreases the benefits of this traditional therapeutic strategy. In particular, bacteria have developed versatile strategies to withstand antibiotic killing, including production of hydrolases such as β-lactamases[2], modifying enzymes such as the colistin resistance enzyme MCR[3] and tigecycline resistance enzymes Tet(X3/X4)[4,5], and activation of bacterial efflux pumps[6]. These phenotypes are mainly mediated by the acquisition and expression of resistance genes in bacteria. Recently, in contrast to gene-mediated antibiotic resistance, a phenomenon termed antibiotic tolerance in bacteria that are genetically susceptible but phenotypically tolerant to antibiotic treatment has been spotlighted[7]. Different from antibiotic resistance, which can be characterized by determination of minimum inhibitory concentration (MIC) and genomic analysis, antibiotic tolerance is not easy to distinguish and is therefore commonly overlooked[8]. This may give an interpretation on the clinical failures that bacteria-active antibiotics screened from in vitro assays could not effectively cure corresponding infections. Moreover, antibiotic tolerance has been proved to facilitate the evolution of resistance[9,10]. Therefore, a deeper understanding of predisposing factors for antibiotic tolerance in vivo is a prerequisite for optimizing therapeutic strategies to combat complicated infectious diseases.

Studies of the long-term administration of HFD in mammals have proposed that a HFD is hazardous for human health and is linked to obesity[11] and diabetes[12]. Furthermore, more evidence has shown that HFD is associated with several other seemingly unrelated diseases. For example, HFD augmented the numbers and function of *Lgr5*$^+$ intestinal stem cells in the mammalian intestine, therefore enhancing their abilities to initiate tumours[13]. Besides, HFD was found to induce brain insulin resistance and cognitive impairment in mice[14]. Meanwhile, it has been widely acknowledged that HFD is correlated with the composition of murine gut microbiota[15,16]; the gut microbiota has been implicated in the regulation of multiple host metabolic pathways as well as human health[17,18]. However, little is known about the correlation between HFD and antibiotic efficacy, and the potential role of gut microbiota in this relationship.

In this Article, we investigated antibiotic killing against multiple pathogens in standard-diet- and HFD-fed mice. Interestingly, we found that HFD decreased antibiotic efficacy in the mouse infection models, which was further proved to be correlated with changes in composition of gut microbiota and related metabolites. Furthermore, our data showed that HFD in mice affected the biosynthesis of IAA, which had a role in the inhibition and eradication of tolerant cells potentially through the activation of bacterial metabolism. Collectively, our findings demonstrate that long-term HFD impairs the efficacy of bactericidal antibiotics by altering the composition and diversity of gut microbiota.

Results

HFD impairs the efficacy of bactericidal antibiotics. To examine the correlation between HFD and antibiotic efficacy in vivo,

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PROVA 14

1. Nelle cellule degli eucarioti, durante il processo catabolico che porta alla demolizione di una molecola di glucosio, in quale fase viene liberato il maggior numero di CO2?

2. Come sono identificate le righe di EXCEL?

REVIEWS

A guide to machine learning for biologists

Joe G. Greener[1,2], *Shaun M. Kandathil*[1,2], *Lewis Moffat*[1] *and David T. Jones*[1]✉

Abstract | The expanding scale and inherent complexity of biological data have encouraged a growing use of machine learning in biology to build informative and predictive models of the underlying biological processes. All machine learning techniques fit models to data; however, the specific methods are quite varied and can at first glance seem bewildering. In this Review, we aim to provide readers with a gentle introduction to a few key machine learning techniques, including the most recently developed and widely used techniques involving deep neural networks. We describe how different techniques may be suited to specific types of biological data, and also discuss some best practices and points to consider when one is embarking on experiments involving machine learning. Some emerging directions in machine learning methodology are also discussed.

Deep learning
Machine learning methods based on neural networks. The adjective 'deep' refers to the use of many hidden layers in the network, two hidden layers as a minimum but usually many more than that. Deep learning is a subset of machine learning, and hence of artificial intelligence more broadly.

Artificial neural networks
A collection of connected nodes loosely representing neuron connectivity in a biological brain. Each node is part of a layer and represents a number calculated from the previous layer. The connections, or edges, allow a signal to flow from the input layer to the output layer via hidden layers.

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Humans make sense of the world around them by observing it, and learning to predict what might happen next. Consider a child learning to catch a ball: the child (usually) knows nothing about the physical laws that govern the motion of a thrown ball; however, by a process of observation, trial and error, the child adjusts his or her understanding of the ball's motion, and how to move his or her body, until he or she is able to catch it reliably. In other words, the child has learned how to catch the ball by building a sufficiently accurate and useful 'model' of the process, by repeatedly testing this model against the data and by making corrections to the model to make it better.

'Machine learning' refers broadly to the process of fitting predictive models to data or of identifying informative groupings within data. The field of machine learning essentially attempts to approximate or imitate humans' ability to recognize patterns, albeit in an objective manner, using computation. Machine learning is particularly useful when the dataset one wishes to analyse is too large (many individual data points) or too complex (contains a large number of features) for human analysis and/or when it is desired to automate the process of data analysis to establish a reproducible and time-efficient pipeline. Data from biological experiments frequently possess these properties; biological datasets have grown enormously in both size and complexity in the past few decades, and it is becoming increasingly important not only to have some practical means of making sense of this data abundance but also to have a sound understanding of the techniques that are used. Machine learning has been used in biology for a number of decades, but it has steadily grown in importance to the point where it is used in nearly every field of biology. However, only in the past few years has the field taken a more critical look at the available strategies and begun to assess which methods are most appropriate in different scenarios, or even whether they are appropriate at all.

This Review aims to inform biologists on how they can start to understand and use machine learning techniques. We do not intend to present a thorough literature review of articles using machine learning for biological problems[1], or to describe the detailed mathematics of various machine learning methods[2,3]. Instead, we focus on linking particular techniques to different types of biological data (similar reviews are available for specific biological disciplines; see, for example, REFS[4–11]). We also attempt to distil some best practices of how to practically go about the process of training and improving a model. The complexity of biological data presents pitfalls as well as opportunities for their analysis using machine learning techniques. To address these, we discuss the widespread issues that affect the validity of studies, with guidance on how to avoid them. The bulk of the Review is devoted to the description of a number of machine learning techniques, and in each case we provide examples of the appropriate application of the method and how to interpret the results. The methods discussed include traditional machine learning methods, as these are still the best choices in many cases, and deep learning with artificial neural networks, which are emerging as the most effective methods for many tasks. We finish by describing what the future holds for incorporating machine learning in data analysis pipelines in biology.

There are two goals when one is using machine learning in biology. The first is to make accurate predictions

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PROVA 15

1. Quando si parla di geni, cosa si intende con locus?

2. Al fine di creare una password il più possibile sicura quale criterio è consigliabile usare?

REVIEWS

Control of satellite cell function in muscle regeneration and its disruption in ageing

Pedro Sousa-Victor[1] ✉, *Laura García-Prat*[2] ✉ *and Pura Muñoz-Cánoves*[3,4,5] ✉

Abstract | Skeletal muscle contains a designated population of adult stem cells, called satellite cells, which are generally quiescent. In homeostasis, satellite cells proliferate only sporadically and usually by asymmetric cell division to replace myofibres damaged by daily activity and maintain the stem cell pool. However, satellite cells can also be robustly activated upon tissue injury, after which they undergo symmetric divisions to generate new stem cells and numerous proliferating myoblasts that later differentiate to muscle cells (myocytes) to rebuild the muscle fibre, thereby supporting skeletal muscle regeneration. Recent discoveries show that satellite cells have a great degree of population heterogeneity, and that their cell fate choices during the regeneration process are dictated by both intrinsic and extrinsic mechanisms. Extrinsic cues come largely from communication with the numerous distinct stromal cell types in their niche, creating a dynamically interactive microenvironment. This Review discusses the role and regulation of satellite cells in skeletal muscle homeostasis and regeneration. In particular, we highlight the cell-intrinsic control of quiescence versus activation, the importance of satellite cell–niche communication, and deregulation of these mechanisms associated with ageing. The increasing understanding of how satellite cells are regulated will help to advance muscle regeneration and rejuvenation therapies.

Maintaining healthy muscle is a major determinant of the quality of life over a lifetime. Skeletal muscle, the largest tissue by mass in the body, regulates posture, voluntary movements, respiration and metabolic functions. This tissue consists of aligned bundles of myofibres (which are multinucleated, striated contractile muscle cells) and an intricate network of blood vessels, nerves and the extracellular matrix (ECM). A unique feature of healthy adult muscle is its ability to fully recover its architecture and contractile functions following both everyday 'wear-and-tear' and acute damage. This ability is due to the presence of stem cells — called satellite cells — in specialized niche microenvironments below the basal lamina and adjacent to the plasma membrane of myofibres[1,2].

In homeostatic muscle, satellite cells are normally in quiescence (a dormant state with a transient cell cycle inhibition). In response to muscle injuries, which can be either minor (such as strains, stretching or exercise) or severe (such as trauma or degenerative muscular diseases), signals from the altered niche activate satellite cells and promote their myogenic activity. Activated satellite cells (ASCs) undergo remarkable energetic shifts for increased biosynthetic activity and cell proliferation that provides sufficient progeny to support regeneration; after several cycles of proliferation, these cells exit the cell cycle and either differentiate and subsequently fuse at the site of injury to repair the damaged fibres or self-renew and reconstitute the quiescent satellite cell (QSC) pool to seed future rounds of muscle damage and repair. Recent studies analysing the time window during which satellite cells stop proliferating after an injury show that the choice to either differentiate or self-renew is influenced by mutual interactions between both cell states through poorly understood mechanisms[3–5]. Also, local injury signals trigger profound changes to the components of the regenerating niche, which influence the satellite cell transitions through the distinct regeneration stages. In addition, injury-released systemic signals such as hepatocyte growth factor (HGF) activator (HGFA)[6] can activate the transition of QSCs to an intermediate primed 'G_{Alert} state', which can enter the cell cycle more rapidly and has a more efficient regeneration process[7]. Once tissue regeneration is complete, skeletal muscle regains homeostasis, satellite cells re-enter quiescence and the mechanisms that operated before the insult are re-established. Satellite cells also activate and proliferate in response to increasing muscle load, such as

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PROVA ESTRATTA

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PROVA 16

1. Cosa sono le gap-junctions?

2. Tra

   Windows e Linux

   Word ed Excel

   Windows e Powerpoint

   Quali sono sistemi operativi?

SP AP

3. Leggere e tradurre

ARTICLES
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nature medicine

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Safety, immunogenicity and efficacy of PfSPZ Vaccine against malaria in infants in western Kenya: a double-blind, randomized, placebo-controlled phase 2 trial

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The radiation-attenuated *Plasmodium falciparum* sporozoite (PfSPZ) vaccine provides protection against *P. falciparum* infection in malaria-naïve adults. Preclinical studies show that T cell-mediated immunity is required for protection and is readily induced in humans after vaccination. However, previous malaria exposure can limit immune responses and vaccine efficacy (VE) in adults. We hypothesized that infants with less previous exposure to malaria would have improved immunity and protection. We conducted a multi-arm, randomized, double-blind, placebo-controlled trial in 336 infants aged 5–12 months to determine the safety, tolerability, immunogenicity and efficacy of the PfSPZ Vaccine in infants in a high-transmission malaria setting in western Kenya (NCT02687373). Groups of 84 infants each received 4.5×10^5, 9.0×10^5 or 1.8×10^6 PfSPZ Vaccine or saline three times at 8-week intervals. The vaccine was well tolerated; 52 (20.6%) children in the vaccine groups and 20 (23.8%) in the placebo group experienced related solicited adverse events (AEs) within 28 d postvaccination and most were mild. There was 1 grade 3-related solicited AE in the vaccine group (0.4%) and 2 in the placebo group (2.4%). Seizures were more common in the highest-dose group (14.3%) compared to 6.0% of controls, with most being attributed to malaria. There was no significant protection against *P. falciparum* infection in any dose group at 6 months (VE in the 9.0×10^5 dose group = −6.5%, $P = 0.598$, the primary statistical end point of the study). VE against clinical malaria 3 months after the last dose in the highest-dose group was 45.8% ($P = 0.027$), an exploratory end point. There was a dose-dependent increase in antibody responses that correlated with VE at 6 months in the lowest- and highest-dose groups. T cell responses were undetectable across all dose groups. Detection of $V\delta2^+V\gamma9^+$ T cells, which have been correlated with induction of PfSPZ Vaccine T cell immunity and protection in adults, were infrequent. These data suggest that PfSPZ Vaccine-induced T cell immunity is age-dependent and may be influenced by $V\delta2^+V\gamma9^+$ T cell frequency. Since there was no significant VE at 6 months in these infants, these vaccine regimens will likely not be pursued further in this age group.

Malaria is a mosquito-borne parasitic disease responsible for an estimated 229 million cases and 409,000 deaths in 2019, primarily from *P. falciparum* among sub-Saharan African children[1]. Between 2000 and 2015 there was a substantial reduction in global malaria cases and deaths due to the implementation of malaria control efforts[2]. However, despite these efforts, since 2015 the number of annual cases and deaths has remained stable, highlighting the urgent need to develop vaccines to prevent malaria.

The most clinically advanced vaccine against *P. falciparum*, RTS,S, is a subunit vaccine consisting of a single recombinant protein, the *P. falciparum* circumsporozoite protein (PfCSP), administered with the adjuvant AS01. In a phase 3 clinical trial in 5–17-month-old infants, three vaccinations with RTS,S/AS01 conferred 51.3% vaccine efficacy (VE) against all episodes of *P. falciparum* clinical disease at one year[3]; 4 vaccinations given over 21 months conferred 36.3% VE over 4 years[4]. Protection by RTS,S is believed to be primarily antibody-mediated[5].

The PfSPZ Vaccine uses a different approach consisting of live (metabolically active), nonreplicating, radiation-attenuated, aseptic, purified, cryopreserved *P. falciparum* sporozoites (SPZ). Initial studies showed an approximate 60–100% VE up to 14 months against homologous (the same parasite strain is used for challenge

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ANTONELLA POTENZA 18/11/2021 PROVA ESTRATTA

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PROVA 17

1. Qual è il ruolo della membrana cellulare?

2. In Microsoft Office per cosa vengono utilizzate le macro?

SP AC

3. Leggere e tradurre

BRIEF COMMUNICATION
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Immunogenicity and reactogenicity of heterologous ChAdOx1 nCoV-19/mRNA vaccination

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Heterologous priming with the ChAdOx1 nCoV-19 vector vaccine followed by boosting with a messenger RNA vaccine (BNT162b2 or mRNA-1273) is currently recommended in Germany, although data on immunogenicity and reactogenicity are not available. In this observational study we show that, in healthy adult individuals ($n = 96$), the heterologous vaccine regimen induced spike-specific IgG, neutralizing antibodies and spike-specific CD4 T cells, the levels of which which were significantly higher than after homologous vector vaccine boost ($n = 55$) and higher or comparable in magnitude to homologous mRNA vaccine regimens ($n = 62$). Moreover, spike-specific CD8 T cell levels after heterologous vaccination were significantly higher than after both homologous regimens. Spike-specific T cells were predominantly polyfunctional with largely overlapping cytokine-producing phenotypes in all three regimens. Recipients of both the homologous vector regimen and the heterologous vector/mRNA combination reported greater reactogenicity following the priming vector vaccination, whereas heterologous boosting was well tolerated and comparable to homologous mRNA boosting. Taken together, heterologous vector/mRNA boosting induces strong humoral and cellular immune responses with acceptable reactogenicity profiles.

Among the currently authorized COVID-19 vaccines, the ChAdOx1 nCoV-19 adenovirus-based vector vaccine (ChAdOx1) and the two mRNA vaccines (BNT162b2 and mRNA-1273) have been the most widely used. Both vaccine types are immunogenic and have shown remarkable efficacy in preventing COVID-19 disease[1–3]. In March 2021, administration of the ChAdOx1 vaccine was temporarily suspended in Germany due to the occurrence of life-threatening cerebral venous thrombosis and thrombocytopenia, primarily in younger women[4,5]. This resulted in revised recommendations for secondary vaccination of all individuals who had received the first dose of the vaccine[6]. Individuals above the age of 60 years are recommended to complete vaccination with the vector vaccine, whereas heterologous boosting with an mRNA vaccine is recommended in those <60 years, with the option to voluntarily remain on a homologous vector regimen[6]. Comparative analyses of immunogenicity between the authorized vaccine regimens are scarce, and knowledge on immunity and reactogenicity after heterologous vaccination is currently limited. We have found that priming with the ChAdOx1 vaccine showed a stronger induction of spike-specific T cell responses as compared to mRNA priming, while antibody responses were more pronounced after mRNA priming[7]. We hypothesized that differences among the vaccine types after priming may influence cellular and humoral immunity following secondary vaccination. We therefore prospectively enrolled three groups of individuals to study the immunogenicity and reactogenicity of a heterologous vector/mRNA prime–booster regimen in comparison to the standard homologous regimens. A detailed analysis of spike-specific IgG levels and neutralizing antibody activity was performed. In addition, spike-specific CD4 and CD8 T cells were characterized using flow cytometry. Adverse events within the first week after the priming and booster doses were self-reported based on a standardized questionnaire.

A total of 216 immunocompetent individuals, primarily comprising employees, were prospectively enrolled at Saarland University Medical Center before secondary vaccination with the authorized vaccines ChAdOx1 nCoV-19, BNT162b2 or mRNA-1273 (Methods). Ninety-seven study participants received heterologous vaccination with the ChAdOx1 vector and mRNA booster (vector/mRNA), whereas 55 and 64 received homologous regimens with vector or mRNA vaccine, respectively (vector/vector and mRNA/mRNA; Extended Data Fig. 1). As per guidelines, the time between primary and secondary vaccination was shorter for mRNA-primed (4.3 ± 1.1 weeks) than for vector-primed individuals, with no difference between vector-based heterologous (11.2 ± 1.3 weeks) and homologous regimens (10.8 ± 1.4 weeks). Blood samples were drawn at a median of 14 (interquartile range (IQR) = 2) days after vaccination. Although all individuals had no known history of SARS-CoV-2 infection, three tested positive for SARS-CoV-2 nucleocapsid (N)-specific IgG and were excluded from further analyses. The groups had similar gender distribution. However, individuals on the homologous vector regimen were slightly older than the two other groups, who were of similar age (Extended Data Fig. 2). Leukocyte counts, including granulocytes, monocytes and lymphocytes, as well as major lymphocyte subpopulations such as CD4 and CD8 T cells, and B cells, did not differ between the groups. This also

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PROVA 18

1. Quando si parla di enzimi cosa s'intende per modello chiave- serratura?

2. In informatica cosa sono i file con estensione .zip?

nature medicine

ARTICLES

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CAR T cells with dual targeting of CD19 and CD22 in pediatric and young adult patients with relapsed or refractory B cell acute lymphoblastic leukemia: a phase 1 trial

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Chimeric antigen receptor (CAR) T cells targeting CD19 or CD22 have shown remarkable activity in B cell acute lymphoblastic leukemia (B-ALL). The major cause of treatment failure is antigen downregulation or loss. Dual antigen targeting could potentially prevent this, but the clinical safety and efficacy of CAR T cells targeting both CD19 and CD22 remain unclear. We conducted a phase 1 trial in pediatric and young adult patients with relapsed or refractory B-ALL ($n = 15$) to test AUTO3, autologous transduced T cells expressing both anti-CD19 and anti-CD22 CARs (AMELIA trial, EUDRA CT 2016-004680-39). The primary endpoints were the incidence of grade 3–5 toxicity in the dose-limiting toxicity period and the frequency of dose-limiting toxicities. Secondary endpoints included the rate of morphological remission (complete response or complete response with incomplete bone marrow recovery) with minimal residual disease-negative response, as well as the frequency and severity of adverse events, expansion and persistence of AUTO3, duration of B cell aplasia, and overall and event-free survival. The study endpoints were met. AUTO3 showed a favorable safety profile, with no dose-limiting toxicities or cases of AUTO3-related severe cytokine release syndrome or neurotoxicity reported. At 1 month after treatment the remission rate (that is, complete response or complete response with incomplete bone marrow recovery) was 86% (13 of 15 patients). The 1 year overall and event-free survival rates were 60% and 32%, respectively. Relapses were probably due to limited long-term AUTO3 persistence. Strategies to improve CAR T cell persistence are needed to fully realize the potential of dual targeting CAR T cell therapy in B-ALL.

CD19 and CD22 chimeric antigen receptor (CAR) T cell therapies have shown promising efficacy in relapsed or refractory B-lineage acute lymphoblastic leukemia (B-ALL). However, CD19-negative relapse is the predominant cause of treatment failure in patients treated with anti-CD19 CAR T cells as a standalone therapy, occurring in 25–42% of responding patients[1,2]. Similarly, reduced CD22 antigen density at relapse was observed in a phase 1 study of anti-CD22 CAR T cell therapy, suggesting that escape by CD22 downregulation is also possible[3,4]. We reasoned that dual antigen targeting may prevent relapse given that a single leukemic stem cell is unlikely to downregulate both CD19 and CD22 simultaneously. Dual CAR targeting can be achieved in different ways: co-administration of two separate CAR T cell products (CD19 CAR T cells and CD22 CAR T cells), co-transduction of T cells with two vectors encoding the two separate CARs, transduction of T cells with a bicistronic vector encoding both CARs, or use of a tandem CAR. The optimal strategy is yet to be defined: each has its strengths and weaknesses and all are currently under investigation[5–7]. Although co-administration or co-transduction allows for the combination of two single targeting vectors with minimal optimization, it results in a heterogeneous mixed product that has a more complex and costly manufacturing procedure. In contrast, bicistronic vectors encoding both CARs, and tandem CAR approaches are cheaper to manufacture and generate a more uniformed homogeneous product that ensures that every transduced cell has the ability to engage both targets.

We developed AUTO3, a CAR T cell treatment with dual specificity generated through transduction of autologous T cells with

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PROVA 19

1. Cosa s'intende per coda poliadenilata?

2. Il suffisso ".pps" di quale è indicativo?

Machinery, regulation and pathophysiological implications of autophagosome maturation

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Abstract | Autophagy is a versatile degradation system for maintaining cellular homeostasis whereby cytosolic materials are sequestered in a double-membrane autophagosome and subsequently delivered to lysosomes, where they are broken down. In multicellular organisms, newly formed autophagosomes undergo a process called 'maturation', in which they fuse with vesicles originating from endolysosomal compartments, including early/late endosomes and lysosomes, to form amphisomes, which eventually become degradative autolysosomes. This fusion process requires the concerted actions of multiple regulators of membrane dynamics, including SNAREs, tethering proteins and RAB GTPases, and also transport of autophagosomes and late endosomes/lysosomes towards each other. Multiple mechanisms modulate autophagosome maturation, including post-translational modification of key components, spatial distribution of phosphoinositide lipid species on membranes, RAB protein dynamics, and biogenesis and function of lysosomes. Nutrient status and various stresses integrate into the autophagosome maturation machinery to coordinate the progression of autophagic flux. Impaired autophagosome maturation is linked to the pathogenesis of various human diseases, including neurodegenerative disorders, cancer and myopathies. Furthermore, invading pathogens exploit various strategies to block autophagosome maturation, thus evading destruction and even subverting autophagic vacuoles (autophagosomes, amphisomes and autolysosomes) for survival, growth and/or release.
Here, we discuss the recent progress in our understanding of the machinery and regulation of autophagosome maturation, the relevance of these mechanisms to human pathophysiology and how they are harnessed by pathogens for their benefit. We also provide perspectives on targeting autophagosome maturation therapeutically.

Cells in multicellular organisms constantly experience diverse stresses, including protein misfolding, organelle damage, scarcity of nutrients/energy and invasion by pathogens. One mechanism exploited by cells to combat stresses is the lysosome-mediated degradation of intracellular components via autophagy. Autophagy involves the formation of an isolation membrane (or phagophore), which further expands and closes to form the double-membrane autophagosome[1–3]. The mechanism of autophagosome formation was excellently reviewed recently[3]. Various cellular materials can be sequestered in autophagosomes, including unselected cytosolic material or selected cargos such as protein aggregates, damaged organelles and pathogens. Following autophagosome closure via membrane abscission, cargos are delivered to the vacuole (yeasts and plants) or to lysosomes (animal cells) (for an overview of the autophagy process see[1–8]; BOX 1). After degradation of the autophagic cargo, the digested content in the autolysosomes is released and lysosomes are re-formed to sustain the autophagic flux (BOX 2). A series of proteins encoded by *ATG* (autophagy-related) genes and *EPG* (ectopic PGL granules) genes — identified mainly from genetic screens in *Saccharomyces cerevisiae* and *Caenorhabditis elegans*, respectively — act at different membrane remodelling steps for autophagosome formation and maturation[3,4,9,10].

In yeast, autophagosomes are formed in the vicinity of and directly fuse with the much larger vacuole, whereas in multicellular organisms, newly formed autophagosomes fuse with different endolysosomal vesicles such as early/late endosomes and lysosomes to form non-degradative, single-membrane structures called 'amphisomes', which gradually acquire

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PROVA 20

1. Cosa è conservato, della vecchia molecola, in una nuova molecola di DNA dopo la duplicazione?

2. A cosa serve il tasto CAPS LOCK sulla tastiera?

Endocytosis in the context-dependent regulation of individual and collective cell properties

Sara Sigismund[1,2], *Letizia Lanzetti*[3,4], *Giorgio Scita*[2,5] *and Pier Paolo Di Fiore*[1,2]✉

Abstract | Endocytosis allows cells to transport particles and molecules across the plasma membrane. In addition, it is involved in the termination of signalling through receptor downmodulation and degradation. This traditional outlook has been substantially modified in recent years by discoveries that endocytosis and subsequent trafficking routes have a profound impact on the positive regulation and propagation of signals, being key for the spatiotemporal regulation of signal transmission in cells. Accordingly, endocytosis and membrane trafficking regulate virtually every aspect of cell physiology and are frequently subverted in pathological conditions. Two key aspects of endocytic control over signalling are coming into focus: context-dependency and long-range effects. First, endocytic-regulated outputs are not stereotyped but heavily dependent on the cell-specific regulation of endocytic networks. Second, endocytic regulation has an impact not only on individual cells but also on the behaviour of cellular collectives. Herein, we will discuss recent advancements in these areas, highlighting how endocytic trafficking impacts complex cell properties, including cell polarity and collective cell migration, and the relevance of these mechanisms to disease, in particular cancer.

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Endocytosis is used by cells to internalize various types of molecules, including nutrients and fluids, which could not otherwise pass through the plasma membrane[1,2]. This has probably represented the initial driving force behind its emergence in evolution, yet the system has been exploited to actively regulate various forms of communication within the cell and between the cell and its environment. Signalling receptors, for instance, are internalized upon engagement by cognate ligands and frequently targeted for degradation in the lysosome, resulting in long-term signalling attenuation[3,4]. In addition, regardless of their interaction with extracellular moieties, many surface-resident molecules (mostly, though not exclusively, proteins) are internalized and either degraded or recycled back to the cell surface, thereby providing a mechanism through which the cell controls and adjusts its repertoire of plasma membrane-resident molecules for various functional purposes. Yet, these are only particular facets of endocytosis, whose impact on cellular homeostasis appears to be much wider (Supplementary Table 1).

A modern view of endocytic trafficking is that of a "vast program, deeply engrained in the cellular masterplan and inextricably intertwined with signaling, which constitutes the major communication infrastructure in the cell"[1]. At the individual cell level, for instance, endosomes represent major signalling stations. This is embodied in the concept of the signalling endosome — a platform capable of sustaining signalling by numerous mechanisms, including the assembly of endosome-specific signalling complexes, crosstalk, regulation of signal persistence in intracellular compartments, and signal computing and resolution in time and space[5]. Endocytosis also controls the execution of polarized cell functions through the redistribution of surface molecules towards sites of polarized activities. In this case, the fast and site-directed redistribution of membrane proteins is not achieved by planar diffusion on the plasma membrane but rather by cycles of endocytosis and directed recycling[6–9].

Although these activities are largely pertinent to the workings of individual cells, it is becoming increasingly clear that cellular collectives are also controlled by endocytosis. This is remarkable, as it entails that endocytic events occurring on the level of a single cell must be synchronized, frequently spanning a distance of hundreds of cells, to contribute to a coordinated behaviour[10–13].

Mechanistically, endocytosis has long been considered a rather stereotyped process, irrespective of the cell type and (to some extent) transported cargo. This vision started to change with the realization that clathrin-coated pits, responsible for clathrin-mediated

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PROVA 21

1. A quale scopo si utilizza la tecnica PCR?

2. In Access cosa è una "Query"?

SP

Gene regulation by long non-coding RNAs and its biological functions

Luisa Statello[1,2,6], *Chun-Jie Guo*[3,6], *Ling-Ling Chen*[3,4,5]✉ *and Maite Huarte*[1,2]✉

Abstract | Evidence accumulated over the past decade shows that long non-coding RNAs (lncRNAs) are widely expressed and have key roles in gene regulation. Recent studies have begun to unravel how the biogenesis of lncRNAs is distinct from that of mRNAs and is linked with their specific subcellular localizations and functions. Depending on their localization and their specific interactions with DNA, RNA and proteins, lncRNAs can modulate chromatin function, regulate the assembly and function of membraneless nuclear bodies, alter the stability and translation of cytoplasmic mRNAs and interfere with signalling pathways. Many of these functions ultimately affect gene expression in diverse biological and physiopathological contexts, such as in neuronal disorders, immune responses and cancer. Tissue-specific and condition-specific expression patterns suggest that lncRNAs are potential biomarkers and provide a rationale to target them clinically. In this Review, we discuss the mechanisms of lncRNA biogenesis, localization and functions in transcriptional, post-transcriptional and other modes of gene regulation, and their potential therapeutic applications.

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Genomes are extensively transcribed and give rise to thousands of long non-coding RNAs (lncRNAs), which are defined as RNAs longer than 200 nucleotides that are not translated into functional proteins. This broad definition encompasses a large and highly heterogeneous collection of transcripts that differ in their biogenesis and genomic origin. Statistics from Human GENCODE suggest that the human genome contains more than 16,000 lncRNA genes, but other estimates exceed 100,000 human lncRNAs[1,2]. These mainly include lncRNAs transcribed by RNA polymerase II (Pol II), but also by other RNA polymerases; and lncRNAs from intergenic regions (lincRNAs) as well as sense or antisense transcripts that overlap with other genes. The resulting lncRNAs are often capped by 7-methyl guanosine (m^7G) at their 5′ ends, polyadenylated at their 3′ ends and spliced similarly to mRNAs (FIG. 1a). It is worthwhile noting that enhancer and promoter regions are also transcribed into enhancer RNAs (eRNAs) and promoter upstream transcripts, respectively[3].

The number of functional lncRNAs is still debated. Although evidence is still lacking to support the functionality of most lncRNAs, thereby rendering them transcription by-products, it is well documented that a growing number of lncRNAs have important cellular functions. The expression of a considerable number of lncRNAs is regulated and some have roles in different mechanisms of gene regulation. Several lncRNAs control the expression of nearby genes by affecting their transcription, and also affect other facets of chromatin biology, such as DNA replication or the response to DNA damage and repair. Other lncRNAs function away from their loci; their functions can be of a structural and/or regulatory nature and involve different stages of mRNA life, including splicing, turnover and translation, as well as signalling pathways. Consequently, lncRNAs affect several cellular functions that are of great physiological relevance, and alteration of their expression is inherent to numerous diseases. The specific expression patterns of these functional lncRNAs have the potential of being used as optimal disease biomarkers, and strategies are under development for their therapeutic targeting.

In this Review, we discuss emerging themes in lncRNA biology, including recent understanding of their biogenesis and their regulatory functions in *cis* and in *trans* at the transcriptional and post-transcriptional levels. We then discuss the pathological consequences of lncRNA dysregulation in neuronal disorders, haematopoiesis, immune responses and cancer. Finally, we discuss how the existing knowledge of lncRNAs allows the development of lncRNA-based therapeutic targeting.

Biogenesis of lncRNAs

Most lncRNA species are transcribed by Pol II. As such, many have 5′-end m^7G caps and 3′-end poly(A) tails, and are presumed to be transcribed and processed similarly to mRNAs. However, recent studies have begun to reveal distinct transcription, processing, export and turnover of lncRNAs, which are closely linked with their cellular fates and functions.

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PROVA 22

1. Quale tecnica si basa sull'utilizzo di nucleotidi modificati (dideossitrifosfato, ddNTPs) per interrompere la reazione di sintesi in posizioni specifiche?

2. La sigla "ICT" è un acronimo. Cosa significa?

SP AC

3. Leggere e tradurre

ARTICLES
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nature immunology

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Skin and gut imprinted helper T cell subsets exhibit distinct functional phenotypes in central nervous system autoimmunity

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Multidimensional single-cell analyses of T cells have fueled the debate about whether there is extensive plasticity or 'mixed' priming of helper T cell subsets in vivo. Here, we developed an experimental framework to probe the idea that the site of priming in the systemic immune compartment is a determinant of helper T cell-induced immunopathology in remote organs. By site-specific in vivo labeling of antigen-specific T cells in inguinal (i) or gut draining mesenteric (m) lymph nodes, we show that i-T cells and m-T cells isolated from the inflamed central nervous system (CNS) in a model of multiple sclerosis (MS) are distinct. i-T cells were Cxcr6$^+$, and m-T cells expressed P2rx7. Notably, m-T cells infiltrated white matter, while i-T cells were also recruited to gray matter. Therefore, we propose that the definition of helper T cell subsets by their site of priming may guide an advanced understanding of helper T cell biology in health and disease.

Organ-specific autoimmune diseases, including MS, are initiated and maintained by the activation of autoreactive T cells in the systemic immune compartment[1]. In MS, autoreactive T cells are likely activated through molecular mimics of autoantigens acquired at body surfaces[2]. However, it is unclear whether and how the anatomical niche, in which an autoreactive T cell is primed, has an impact on the recruitment, topology and inflammatory effector function of T cells in remote tissues like the CNS. Particularly in MS, the topology and the type of lesions within the CNS are critical determinants of the clinical phenotype[3], and we hypothesize that lesion development in MS is not random but controlled by immune system intrinsic molecular cues, which in turn might already be imprinted at the site of T cell priming outside the CNS. For instance, the gut microbiome might dictate the susceptibility to and severity of autoimmune diseases[4,5], for example, by controlling the adjuvanticity in local lymph nodes, thus inducing distinct helper T cell species.

Helper T cell subsets have been defined by their signature cytokines interferon (IFN)-γ (T_H1), interleukin (IL)-4 (T_H2) and IL-17 (T_H17). This concept has been widely accepted (also in T cell-mediated autoimmune diseases) because cytokines determine the interaction of T cells with other immune cells and thus their function in host defense and inflammation[6]. While these T cell subsets are well characterized on the molecular level after in vitro differentiation of naive T cells[7], a large overlap between T cell subsets has been observed in vivo and and is explained by the plasticity of helper T cells previously 'cleanly' committed to a specific T cell lineage in mice and humans[8,9]. Alternatively, it is an emerging concept that T cell subsets might be primed as mixed phenotypes from the get-go[10,11].

Here, we label antigen-specific T cells in skin draining inguinal lymph nodes (iLN) and gut draining mesenteric lymph nodes (mLN) in vivo, thus establishing a 'provenance-mapping system' for the T cells that are later collected in remote non-lymphoid tissues, such as in the CNS, during the course of experimental autoimmune encephalomyelitis (EAE), a preclinical disease model for MS. We characterize activated T cells defined by their anatomical origin in iLN (i-T cells) or mLN (m-T cells) and provide evidence that the priming site is a fundamental determinant of their commitment to a defined helper T cell lineage. Such imprinting ultimately results in the generation of specific effector functions in the CNS, including white matter versus gray matter infiltration. We show that this concept of 'anatomically' defined helper T cell subsets is compatible with some aspects of the extensive molecular characterization

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PROVA 23

1. In una reazione di PCR esiste un parametro che porterebbe ad una differenza nel risultato se viene variato, quale?

2. A cosa serve la sequenza di caratteri denominata URL?

3. Leggere e tradurre

nature methods

ARTICLES

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Structured illumination microscopy with noise-controlled image reconstructions

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Super-resolution structured illumination microscopy (SIM) has become a widely used method for biological imaging. Standard reconstruction algorithms, however, are prone to generate noise-specific artifacts that limit their applicability for lower signal-to-noise data. Here we present a physically realistic noise model that explains the structured noise artifact, which we then use to motivate new complementary reconstruction approaches. True-Wiener-filtered SIM optimizes contrast given the available signal-to-noise ratio, and flat-noise SIM fully overcomes the structured noise artifact while maintaining resolving power. Both methods eliminate ad hoc user-adjustable reconstruction parameters in favor of physical parameters, enhancing objectivity. The new reconstructions point to a trade-off between contrast and a natural noise appearance. This trade-off can be partly overcome by further notch filtering but at the expense of a decrease in signal-to-noise ratio. The benefits of the proposed approaches are demonstrated on focal adhesion and tubulin samples in two and three dimensions, and on nanofabricated fluorescent test patterns.

SIM is a super-resolution technique that offers twofold increased spatial resolution along two or three dimensions plus optical sectioning with strongly enhanced contrast compared with conventional widefield fluorescence microscopy using uniform illumination[1–6], and is compatible with live cell imaging[7–9]. Further reduction of resolution below the 100 nm length scale can be achieved by making use of the nonlinear fluorescence response[10–13]. Current image reconstruction methods[3,4,14–16] depend on ad hoc tuneable parameters and are susceptible to various types of artifact[17,18]. The effort to distinguish 'real' structural resolution improvement from noise-related deconvolution artifacts in SIM has recently inspired much controversy in the field[19,20]. The root causes of this confusion are (1) the lack of insight into the mechanism of image formation that requires a reconstruction procedure of considerable mathematical complexity, (2) the use of ad hoc parameters with large effect on the final image but with unclear physical meaning and (3) the absence of an unambiguous method to separate true signal from noise.

Here we address these issues first by an in-depth analysis of the propagation of noise through the image reconstruction chain of state-of-the-art linearly filtered SIM. We use this to explain the structured noise artifact of SIM, the amplification of noise structures at intermediate length scales at low signal conditions. In particular, the analysis clarifies the role of ad hoc filtering operations such as regularization. In a next step, the understanding of noise propagation is applied in new SIM reconstruction methods. These methods are designed for optimizing contrast or a natural noise appearance, and to eliminate ad hoc reconstruction parameters. The underlying goal is to make the representation of objects in SIM images as objective as possible.

Results

Propagation of noise through the image reconstruction chain of SIM. The goal of image reconstruction is to provide an estimate e_k^{rec} (with $k = 1, \cdots, N$ the index denoting the different pixels) of the underlying fluorescent object f_k. This estimate depends linearly on the images acquired for the M_r different rotations and M_t different translations of the illumination pattern via a sequence of Fourier (spatial frequency) space manipulations (Supplementary Note 1). The reconstructed object e_k^{rec} is a sum of a term e_k that corresponds to the reconstruction in the absence of noise and a perturbation δe_k of that ideal reconstruction due to noise. The Fourier transforms (FTs) of e_k and δe_k are denoted as $\hat{e}_j$ and $\delta\hat{e}_j$, respectively, where the hat indicates the FT and the index j indicates the Fourier pixel with spatial frequency $\bar{\mathbf{q}}_j$.

The signal power and the noise variance in Fourier space $\hat{S}_j \equiv |\hat{e}_j|^2$ and $\hat{N}_j \equiv \langle|\delta\hat{e}_j|^2\rangle$ are used here to quantify the noise and signal level. The noise variance can be determined empirically by making K repeated acquisitions of a fixed specimen, and computing the variance over the K reconstructions in Fourier space. We have analyzed the propagation of noise through the reconstruction chain (full theory in Supplementary Note 2) and found analytical expressions for the signal power and noise variance:

$$\hat{S}_j = \frac{\hat{A}_j^2 \hat{D}_j^2}{\left[\hat{w}_j + \hat{D}_j\right]^2} \left|\hat{f}_j\right|^2 \tag{1}$$

$$\hat{N}_j = \frac{\hat{A}_j^2}{\left[\hat{w}_j + \hat{D}_j\right]^2} \left(\hat{f}_0 \hat{V}_j + N\sigma^2 \hat{D}_j\right) \tag{2}$$

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10_CONCORSO PUBBLICO, PER TITOLI ED ESAMI, PER LA COPERTURA A TEMPO DETERMINATO, DELLA DURATA DI CINQUE ANNI PER N. 1 POSTO DI COLLABORATORE PROFESSIONALE DI RICERCA SANITARIA - CAT. D, DA ASSEGNARE ALLA UOC NEUROLOGIA 4 – NEUROIMMUNOLOGIA E DELLE MALATTIE NEUROMUSCOLARI

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T cell antigen discovery

Alok V. Joglekar[1,2,3] ✉ and Guideng Li[4,5,6] ✉

T cells respond to threats in an antigen-specific manner using T cell receptors (TCRs) that recognize short peptide antigens presented on major histocompatibility complex (MHC) proteins. The TCR–peptide–MHC interaction mediated between a T cell and its target cell dictates its function and thereby influences its role in disease. A lack of approaches for antigen discovery has limited the fundamental understanding of the antigenic landscape of the overall T cell response. Recent advances in high-throughput sequencing, mass cytometry, microfluidics and computational biology have led to a surge in approaches to address the challenge of T cell antigen discovery. Here, we summarize the scope of this challenge, discuss in depth the recent exciting work and highlight the outstanding questions and remaining technical hurdles in this field.

The challenge of T cell antigen discovery

T cells play a critical role in the elimination of pathogens and the surveillance of pathological cells. They have the ability to discriminate between self- and non-self-antigens via a surface TCR. Most human T cells express classical TCRs, composed of TCRα and TCRβ chains that are co-expressed with CD3 chains (γ, δ, ε and ζ) (Fig. 1a). TCRα and TCRβ chains each consist of a variable (V) and a constant (C) region[1]. The Vα chain is encoded by V and joining (J) gene segments (*TRAV* and *TRAJ*), whereas the Vβ chain is composed of V, diversity (D) and J gene segments (*TRBV*, *TRBD* and *TRBJ*). Multiple copies of each gene segment are encoded in the genome, and are combinatorically rearranged in V–J or V–D–J order to generate diversity in TCRα and TCRβ chains, respectively (Fig. 1b). Additional diversity is generated by non-templated nucleotide (N) additions and/or deletions at gene junctional boundaries. Three complementarity-determining regions (CDRs) located within the V domain of a TCR account for its main interaction with the peptide–MHC (pMHC) complex, thereby conferring antigen specificity on the TCR[1,2]. Recent results from a high-throughput sequencing study suggest that the actual diversity of the TCR repertoire is likely in the range of 10^{11}–10^{12} unique TCRs[3]. Upon TCR recognition of cognate pMHC complexes, the immunoreceptor tyrosine-based activation motifs (ITAMs) in CD3 chains are immediately phosphorylated, triggering signaling cascades that result in T cell activation and induction of T cell function[1,4].

TCR–pMHC interaction is a crucial element of adaptive immunity. Classical $CD8^+$ and $CD4^+$ αβT cells recognize short peptides presented by the MHC class I and II (MHC I and II) molecules, respectively[5]. In striking contrast to these MHC-restricted αβT cells, unconventional γδT cells composed of TCRγ and TCRδ chains can recognize non-peptide antigens (for example, lipid antigens) in a MHC-unrestricted manner[6]. For the purposes of this article, we will focus on the antigens recognized by αβTCRs. Peptide epitopes can be displayed on the cell surface, through either the cytosolic pathway (MHC-I-restricted epitopes) or the endocytic pathway (MHC-II-restricted epitopes)[7,8]. The avidity of the TCR–pMHC interaction is enhanced by the binding of the co-receptors CD4 and CD8 to MHC II and MHC I, respectively[9]. Another feature of TCR–pMHC interactions is promiscuity: each TCR is capable of recognizing millions of different peptides, and numerous different TCRs can recognize a given peptide[10–12]. However, technical limitations have prevented extensive mapping of the landscape of TCR–pMHC interactions. Methods that allow reliable identification of TCR ligands would address this unmet need and greatly aid the fields of autoimmunity, infectious diseases and cancer immunology.

The challenge of understanding the molecular rules governing TCR–pMHC interaction is complicated by four main factors. First, T cells specific for a given antigen are presented in the peripheral blood at a very low frequency, making it hard to accurately detect these rare antigen-specific populations[13]. Second, both TCRs and pMHCs are polyspecific[10–12,14], allowing recognition of peptides with minimal sequence similarity[15]. Third, the affinities of TCR–pMHC interactions are several orders of magnitude lower than those of antibodies for their ligands[16], requiring sensitive biochemical techniques for detection. Fourth, the processing and presentation of antigens produce large numbers of potential T cell epitopes, making difficult to synthesize or express them in antigen-presenting cells (APCs) for screening. Recently, several in silico tools, such as NetMHC, have been developed to predict MHC binding, but these still cannot accurately predict the immunogenicity of these peptides[17–19].

Over the past few years, extensive progress has been made in the field of T cell antigen discovery, enabling the characterization of T cell responses to tumors, pathogens, self-antigens and food-related antigens. Here, we review recent advances in TCR ligand discovery, discuss their strengths and limitations, and highlight the outstanding challenges in this field.

Antigen-directed approaches

Various antigen-directed approaches have been developed to characterize antigen-specific T cells (Fig. 2). The classic strategy is to detect TCR–pMHC interactions by assessing the functional response of T cells after exposure to given antigens. These methods include measurement of T cell proliferation, detection of antigen-specific cytolytic activity of T cells by chromium release, ELISpot assay and intracellular cytokine staining[13,20–24]. Pioneering work using this approach to study antitumor immunity was conducted by co-culturing selected T cell clones with antigen-negative

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Human small intestinal infection by SARS-CoV-2 is characterized by a mucosal infiltration with activated CD8$^+$ T cells

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The SARS-CoV-2 pandemic has so far claimed over three and a half million lives worldwide. Though the SARS-CoV-2 mediated disease COVID-19 has first been characterized by an infection of the upper airways and the lung, recent evidence suggests a complex disease including gastrointestinal symptoms. Even if a direct viral tropism of intestinal cells has recently been demonstrated, it remains unclear, whether gastrointestinal symptoms are caused by direct infection of the gastrointestinal tract by SARS-CoV-2 or whether they are a consequence of a systemic immune activation and subsequent modulation of the mucosal immune system. To better understand the cause of intestinal symptoms we analyzed biopsies of the small intestine from SARS-CoV-2 infected individuals. Applying qRT-PCR and immunohistochemistry, we detected SARS-CoV-2 RNA and nucleocapsid protein in duodenal mucosa. In addition, applying imaging mass cytometry and immunohistochemistry, we identified histomorphological changes of the epithelium, which were characterized by an accumulation of activated intraepithelial CD8$^+$ T cells as well as epithelial apoptosis and subsequent regenerative proliferation in the small intestine of COVID-19 patients. In summary, our findings indicate that intraepithelial CD8$^+$ T cells are activated upon infection of intestinal epithelial cells with SARS-CoV-2, providing one possible explanation for gastrointestinal symptoms associated with COVID-19.

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INTRODUCTION

In December 2019, the disease known as Coronavirus Disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was reported for the first time and has by now been claimed over three and a half million lives around the world[1]. COVID-19 is mainly characterized by symptoms caused by a viral infection of the upper airways and the lung such as fever, cough, dyspnea, anosmia, and fatigue[2]. However, an increasing number of reports indicate that COVID-19 is more of systemic nature, including cardiovascular, haematological, renal, neurologic, and dermatologic as well as gastrointestinal manifestations[3]. To date it remains unknown, whether these effects are caused by direct SARS-CoV-2 infection of the respective tissues[4–7], indirectly by the excessive release of cytokines[8], by thromboembolic effects impairing microcirculation[3], or by a combination of all mentioned complications.

Gastrointestinal involvement, likely including virus replication, has already been reported for other coronaviruses, including SARS-CoV[9], the Middle East Respiratory Syndrome (MERS)[10] and the four endemic human Coronaviruses[11]. In a recent meta-analysis, 17.6% of patients suffering from COVID-19 reported gastrointestinal symptoms such as loss of appetite, nausea/vomiting, diarrhea, and/or abdominal pain/discomfort[12]. In the same study, the pooled prevalence of stool samples positive for SARS-CoV-2 RNA was 48.1%, of which 70.3% of the samples remained positive for viral RNA even when respiratory specimens were tested negative for viral RNA[12]. Recently, SARS-CoV-2 RNA was not only shown to be present in feces but also in esophageal,

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