Progress in Inflammation Research 91
Series Editors: Michael J. Parnham · Thorsten J. Maier
Emanuela Ricciotti · Carmela Matrone

Francis Man
Simon J. Cleary *Editors*

Imaging Inflammation

Springer

Progress in Inflammation Research

Volume 91

This book series addresses all key topical aspects of basic research, therapy and its clinical implications in the field of inflammatory diseases. It provides a unique reference source for academic and industrial biomedical researchers, drug development personnel, immunologists, rheumatologists, cardiologists, allergologists and many other relevant clinical disciplines. Each publication supplies regular scientific updates on newest developments and allow providing access to state-of-the-art techniques and technologies.

The series gathers knowledge from leading authorities on the multiple facets of inflammation research, making it a valuable asset for advanced students in biomedical sciences, early career investigators and for professionals in both basic and translational research and in the clinic. Each volume comprises a carefully selected collection of high-quality review articles on the respective field of expertise. They also introduce new investigators to the most pertinent aspects of inflammatory disease and allow established investigators to understand fundamental ideas, concepts and data on sub-fields that they may not normally follow.

Thus chapters should not comprise extensive data reviews nor provide a means for authors to present new data that would normally be published in peer-reviewed journals. Instead, the chapters should provide a concise overview and guide to the most pertinent and important literature, thus reflecting a conceptual approach rather than a complete review of the particular field of research. Moreover, each chapter should be intelligible for less experienced researchers or even newcomers to the fields of pathology, mechanisms and therapy of inflammatory disease. To this end, authors should consider introducing PhD students or postdocs who are new to the laboratory to the major concepts and the most critical literature in their chosen field of research.

Francis Man • Simon J. Cleary
Editors

Imaging Inflammation

Springer

Editors
Francis Man
Institute of Pharmaceutical Science
King's College London
London, UK

Simon J. Cleary
Department of Medicine
University of California, San Francisco
San Francisco, CA, USA

ISSN 1422-7746 ISSN 2296-4525 (electronic)
Progress in Inflammation Research
ISBN 978-3-031-23660-0 ISBN 978-3-031-23661-7 (eBook)
https://doi.org/10.1007/978-3-031-23661-7

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Compliance with Ethical Standards

Funding: *Francis Man has received salary support from the Sackler Institute of Pulmonary Pharmacology at King's College London. Simon Cleary received salary support from an American Society of Transplantation/CSL Behring Fellowship Basic Science Research Grant and a National Blood Foundation Early-Career Scientific Research Grant from the Association for the Advancement of Blood & Biotherapies.*

Conflict of Interest: *Francis Man is an employee and shareholder of GE HealthCare. The views expressed are his own and not necessarily those of GE HealthCare. Simon Cleary has no financial conflicts of interest.*

Ethical Approval: *This chapter does not involve studies with human participants or animals.*

Preface

We are no longer entirely reliant on assessments of redness, swelling and exudate to identify inflammation by sight—many techniques are now available for molecular imaging of inflammatory responses. New imaging techniques are rapidly being adopted for use in immunology research, and these methods rarely completely supersede existing approaches. This leaves those of us interested in imaging inflammatory responses with a complex choice of which imaging method or methods to use for solving the problem at hand.

We compiled this volume to give researchers interested in the biology of inflammation and immunity a broad guide to some of the imaging techniques that are likely most useful for use in answering their research questions. The readers will find in each chapter of *Imaging Inflammation* a brief introduction to the technical aspects of the respective imaging modalities, followed by examples and discussions of their application in inflammation research.

Imaging Inflammation begins with a historical perspective on imaging on immune responses from Doreen Lau, taking us on a journey from the first observations of living cells by Antonie van Leeuwenhoek to non-invasive whole-body imaging of immune cells by positron emission tomography.

The book chapters then follow an approximate scale that starts from the largest field of view, with whole-body molecular imaging modalities, and progressively narrows down to high-resolution techniques which enable spatial visualisation of gene transcription patterns.

Nuclear imaging modalities are addressed by George Keeling and Francis Man, beginning with brief descriptions of the principles of single-photon emission computed tomography (SPECT) and positron emission tomography (PET) and the various structural classes of radiotracers. This chapter provides an overview of the use of nuclear imaging for the detection and monitoring of inflammatory diseases in patients. It then covers molecular processes involved in inflammation that have more recently become imaging targets and are mostly at the preclinical research stage, including amino acid transporters, fibroblast activation protein and markers of specific subsets of immune cells.

Magnetic resonance imaging (MRI) is another imaging modality that allows whole-body imaging as well as more organ-focused imaging. Here, Vanessa Johanssen, Niloufar Zarghami and Nicola Sibson provide their insights on MRI of neuroinflammation, with a focus on use of magnetic iron nanoparticles targeted towards markers of vascular inflammation and immune cell activation.

Matthew Muller, Jonathan Lindner and Matthew Hagen offer the reader their perspective of ultrasound imaging, using for example B-mode, Doppler, and contrast-enhanced ultrasound to explore rheumatoid arthritis, atherosclerosis and myocardial ischaemia. They demonstrate how targeted microbubbles can enable imaging of specific molecular processes non-invasively, using low-cost handheld probes.

Optical imaging is a modality crossing through a range of scales and can be used for whole-body imaging, tissue-level and subcellular imaging. In their chapter, Jen-Chieh Tseng and Jeffrey Peterson describe the use of chemiluminescence and near-infrared fluorescence for whole-body imaging of inflammation. In particular, they explore the use of reactive probes that emit light in response to the production of reactive oxygen species in inflamed tissues, as well as activatable probes that contain peptide sequences uniquely cleaved by inflammatory proteases such as cathepsins. Perhaps less well known but no less fascinating is the possibility of 3D in vivo fluorescence imaging, which is also briefly covered.

Photoacoustic imaging is a relatively recent imaging modality that combines an optical input signal with an ultrasonic output, combining advantages of both techniques. In this chapter by Jingqin Chen, Zhihua Xie, Liang Song, Xiaojing Gong and Chengbo Liu, readers can learn about label-free photoacoustic imaging of endogenous molecules, such as haemoglobin, and activatable probes that respond to changes in the inflammatory microenvironment. An interesting feature of photoacoustic imaging is its ability to image at different scales depending on instrument configuration, ranging from photoacoustic microscopy that images at high resolutions (a few microns laterally) but limited depths (a few millimetres) to photoacoustic tomography that can image tissues several centimetres deep but at more modest spatial resolution (a few hundred microns). The optical component also enables multispectral imaging, meaning that probes and endogenous molecules that absorb at different wavelengths can be imaged simultaneously and discriminated. This allows multiple inflammatory processes to be captured concomitantly.

Marco De Donatis, Frédéric Fercoq and Leo Carlin explore the use of intravital microscopy to image inflammation in living tissues, tracking single cells as they migrate between blood vessels and organs. In particular, they highlight how intravital microscopy allows the study of systems that are difficult to model *in vitro* because of their complexity, such as host–parasite interactions and viral infections. This chapter gives an update on the latest intravital microscopy approaches, which allow imaging of multiple markers at the same time with high temporal and spatial resolution. These features allow precise determination of cell migration, plasma extravasation and tissue remodelling in live tissues.

Finally, Kenneth Hu takes the reader to the frontier where imaging meets transcriptomics, describing how the spatial organisation of tissues can be mapped

with the low bias and high dimensionality offered by mRNA sequencing. With spatial transcriptomic techniques such as multiplexed fluorescent *in situ* hybridisation, Slide-seq and ZipSeq, analysis is no longer limited to single-digit numbers of targets per sample and transcriptomes can be related to their locations of expression.

Some emerging approaches for imaging that we think will be of future interest to immunologists are not yet covered in this series. These methods include imaging mass spectrometry, imaging mass cytometry, techniques for immunofluorescence imaging of many proteins in the same sample including histo-cytometry, electron and super-resolution microscopy approaches, lattice light sheet imaging and tissue clearing and expansion approaches for sample preparation.

We would like to stress is that there is no 'magic bullet' imaging modality combining the ideal characteristics of a whole-body field-of-view with high spatio-temporal resolution, high sensitivity, unlimited depth of penetration, unbiased imaging of all molecular contents of samples, zero toxicity and low cost. To efficiently advance our fields, it is therefore imperative on us as researchers to choose the techniques we use for our problems wisely and to work on improving methods for continuing progress in inflammation research and beyond.

London, UK — Francis Man
San Francisco, CA, USA — Simon J. Cleary

Acknowledgements

The editors would like to thank Prof. Mike Parnham for the invitation to shape this volume and the authors and reviewers who gave time to produce and refine its contents.

Contents

About the Editors

Francis Man was until recently a postdoctoral scholar at King's College London, where he developed novel PET radiotracers for tracking therapeutic cells and investigated methods for imaging lung epithelial permeability. He has a PhD in Chemical Biology from King's College London and a PharmD from the University of Strasbourg. Francis is currently a Senior Scientist at GE Healthcare, in charge of the preclinical development of imaging agents.

Simon J. Cleary is a postdoctoral scholar at UCSF where he studies mechanisms of acute lung injury and develops new approaches for lung imaging under the mentorship of Mark Looney. He has a PhD in Pharmacology and a BSc in Physiology & Pharmacology from King's College London. Simon was recently awarded an early career grant from the National Blood Foundation for his research into transfusion reactions and organ transplant rejection.

Imaging Inflammation: A Historical Perspective

Doreen Lau

Abstract Inflammation is the body's response to invading pathogens, foreign particles, and abnormal cells. It is a complex set of processes orchestrated by numerous cell types and chemical signalling cascades during disease and injury. Imaging offers direct visual evidence of the cellular and molecular processes involved in inflammation. The five cardinal signs of inflammation—*calor* (heat), *dolor* (pain), *rubor* (redness), *tumor* (swelling), *penuria* (loss of function)—can be felt or seen, but identifying the exact mechanisms underlying these responses and their stimuli has required technological developments in biomedical imaging. Imaging has played a huge role in building our understanding of inflammatory processes and how these sequences of events are involved in inflammatory changes in human health and disease. This review is intended to provide a brief, and by no means complete, historical overview of imaging in inflammation and as a prelude to the subsequent chapters by other authors who will discuss the technicalities and application of advanced imaging techniques.

Keywords Imaging inflammation · Germ theory · Humoral and Cell-mediated immunity · Microscopy · CT · SPECT · PET · Ultrasound · Photoacoustic imaging · MRI

If I have seen further, it is by standing upon the shoulders of giants.
Sir Isaac Newton (1675)

D. Lau (✉)
Oxford Centre for Immuno-Oncology, Nuffield Department of Medicine, University of Oxford, Oxfordshire, UK
e-mail: donc0882@ox.ac.uk

F. Man, S. J. Cleary (eds.), *Imaging Inflammation*, Progress in Inflammation Research 91, https://doi.org/10.1007/978-3-031-23661-7_1

1 Microscopy for Imaging Inflammation: The Germ Theory

The history of imaging inflammation began in the seventeenth century when Antonius van Leeuwenhoek (1632–1723) made his first observation of unicellular microorganisms under the microscope (Fig. 1). Known as the "Father of Microbiology," van Leeuwenhoek was one of the first microscopists to discover the existence of microorganisms [1]. Raised in Delft in what was then known as the Dutch Republic,

Fig. 1 Illustrations from the works of Antonius van Leeuwenhoek, one of the world's first microscopists to examine the details of unicellular life. (**a**) Portrait of van Leeuwenhoek holding his simple microscope. From *Antonius van Leeuwenhoek. Mezzotint* by J. Verkolje, 1686. Public Domain Mark. Wellcome Collection. (**b**) An illustration on the design of van Leeuwenhoek's single-lens microscopes by Henry Baker. From *Arcana naturae detecta / Ab Antonio van Leeuwenhoek, 1695*. Wellcome Collection. (**c**) A replica of van Leeuwenhoek's simple microscope. From *Leeuwenhoek simple microscope (copy)*, Leyden, 1901–1930. Science Museum, London. Wellcome Collection. (**d**) Drawings from van Leeuwenhoek's notes, depicting the size, shape, and movement of bacteria in a dental plaque sample observed under the microscope. From *Arcana naturae detecta/Ab Antonio van Leeuwenhoek*. (**b**, **c**, **d**) Reproduced with permission under CC-BY-4.0 licence (https://creativecommons.org/licenses/by/4.0/). Credit: Wellcome Collection

van Leeuwenhoek was a cloth merchant running a draper's shop when he developed an interest in lens-making, as he wanted a better tool to examine the thread counts and quality of linen and other fabrics better than the magnifying lenses of his time. Van Leeuwenhoek was a great admirer of Robert Hooke's *Micrographia* (1665) when he began making his own improvements to the microscope. He made more than 500 simple microscopes in his lifetime, using crafting methods that he kept as trade secrets. Each microscope consisted of single convex lens held in a silver or copper frame with very short focal length, a magnification factor of about 275 times, and could resolve objects as small as 1 micrometre [2]. Although van Leeuwenhoek was largely self-taught with no formal scientific training, his skills of careful observation and great patience enabled him to make scientific discoveries of fundamental importance and demonstrate the existence of unicellular life. In 1674, he was the first to see this unimagined world of bacteria and protozoa using his single-lens microscopes. He curiously termed the moving objects as *animalcules*, or tiny animals, and accurately described their structures, size, and behaviour [3]. In letters written to the Royal Society of London between 1681 and 1683, van Leeuwenhoek described and illustrated the different types of bacteria present in dental plaque samples. He compared his own oral and faecal microbiota and recorded the morphological differences between bacteria found in different body sites and between health and disease [4]. These discoveries made under the light microscope provided the first direct observation of bacteria, supporting early claims about the germ theory and the existence of disease-causing microorganisms [1]. The light microscope would remain the workhorse for imaging microorganisms until the 1930s, when transmission electron microscopy (TEM) was invented by Ernst Ruska (1906–1988). TEM uses electrons of very short wavelength instead of visible light as the source of illumination and heavy metal staining techniques to produce high resolution images of microorganisms. It provides superior resolving power that captures detailed subcellular structures of bacteria and magnifies the nanometre sizes and geometrical shapes of viruses compared to the micrometre scale detected by light microscopy. Morphological differences between viruses that caused infectious diseases in those days, e.g., the smallpox and chickenpox viruses, have been demonstrated using electron microscopy [5].

In addition to his contributions to the field of microbiology, van Leeuwenhoek performed one of the earliest intravital microscopy experiments, which confirmed William Harvey's theory on blood circulation (1628). Using a special aquatic microscope he had designed, and small live eels mounted in water through a glass tube, van Leeuwenhoek observed the movement of erythrocytes through the capillaries in the tail fins [6]. He demonstrated the transition of blood from the arterial to the venous system and depicted the size and shape of *red corpuscles*, i.e., erythrocytes. He went on to describe and measure erythrocytes and their nuclei in other fishes, amphibians, and mammals, despite not fully understanding the function of blood transportation and the role of microcirculation in inflammation [7]. Van Leeuwenhoek meticulously dissected his specimens with an open razor into thin sections and carefully enclosed them in small paper packets and into larger envelopes attached with his letters to the Royal Society for peer review. He would make

every effort not to introduce tiny animals from any other sources into his experiments and only used snow water which was the purest water available then to replenish evaporated water containing the microorganisms he was examining as he was acutely aware of contamination issues. To provide a measurement scale for this invisible new world, he developed a practical system of micrometry for describing the size of the unicellular organisms he observed under the microscope: "bacteria in pepper-water (2–3 μ), a human erythrocyte (7.2 μ), a hair from his beard (100 μ), and a grain of coarse sand (870 μ)." By the time of his death in 1723, van Leeuwenhoek had written at least 190 letters to the Royal Society detailing his observations of the *animalcules*, which still hold largely true today.

2 Imaging Leukocyte Function in Humoral and Cell-Mediated Immunity

Microscopes have been integral to inflammation research ever since, with generations of intravital microscopists peering curiously into the invisible world to examine the wonders of nature. Henri Dutrochet (1776–1847), a French botanist and physiologist best known for his work on osmosis and contributions to the cell theory, was the first to report his microscopic observations of leukocytes in 1824 [8]. Dutrochet used a simple microscope for his experiments on plant and animal tissues, instead of a compound microscope as the instrument was practically useless in those days due to chromatic aberration and mechanical instability [9]. He described seeing the movement of *vesicular globules*, i.e., leukocytes, through the transparent mucous membrane of living frogs whereby *masses of them surround both the blood vessels* and migrated into tissues during acute inflammation [9]. In 1839, the German anatomist Rudolf Wagner (1805–1864) provided the first detailed drawing of leukocyte rolling in blood vessels [10]. Described as *lymph-corpuscles*, these leukocytes were seen moving more slowly than other blood cells and in close contact with the vessel wall in the webbed feet of a grass frog.

Studies using intravital microscopy techniques were vital in determining the function of leukocytes. During the nineteenth century, research by Julius Cohnheim (1839–1884), Paul Ehrlich (1854–1915), and Élie Metchnikoff (1845–1916) contributed to major discoveries through the use of microscopy in studying inflammation and to the establishment of fundamental concepts in immunology. At the Pathological Institute in Berlin in the 1860s, Julius Cohnheim, a German-Jewish pathologist and skilled microscopist, was conducting extensive research on the causes of inflammation with the "Father of Modern Pathology," Rudolf Virchow (1821–1902), when he visualised the process of leukocyte transmigration in blood vessels. Cohnheim served briefly as a surgeon with the German Army during the Second Schleswig War against Denmark (1864) treating injured soldiers before returning to Berlin to become the chief assistant to Virchow. He published several papers over the years, most notably *Über Entzündung und Eiterung* (*On*

Inflammation and Suppuration) in 1867 which described his microscopic observations of vasodilation, leakage of plasma, and migration of leukocytes at the site of tissue injury during acute inflammation. He further proved the origin of pus as debris formed by the emigration of leukocytes, solving a medical mystery that had puzzled doctors for centuries [11]. In his *Vorlesungen über allgemeine Pathologie* (*Lectures on General Pathology*) in 1889, Cohnheim gave a detailed description of the leukocyte adhesion cascade seen under his microscope through the foot webs and tongues of frogs: "First, in a vein with typical margination of white blood cells, one sees a pointed edge in the outer vessel wall. This moves further away to the outside and if finally connected only through a thin, long stalk. Finally, this stalk is detached, and now a colourless, matte-shining, contractile corpuscle is sitting outside, a white blood cell." With great foresight, he hypothesised that molecular changes in the vascular endothelium may have allowed the *white blood corpuscles* to cross the capillary walls and migrate into tissues [12, 13]. Similar observations were made by others and depicted in Fig. 2.

In the 1870s, Karl Weigert, a German-Jewish pathologist, began assisting Julius Cohnheim in much of his microscopy work. He developed methods for staining bacteria with aniline dyes in histology and bacterial diagnostics, as bacteria were difficult to visualise with the microscope in those days [15]. Karl Weigert was a great influence on his cousin Paul Ehrlich (Fig. 3). As a young schoolboy (and a man soon to become the future Nobel Prize laureate), Ehrlich had always been fascinated by the process of tissue staining for microscopy and therefore decided to continue the research started by his cousin by spending the eighth semester of his medical school perfecting the use of the red dye dahlia (monophenylrosaniline) for histological staining [16]. In 1878, Ehrlich completed his doctorate under Julius Cohnheim at Leipzig University with a dissertation entitled *Beiträge zur Theorie und Praxis der Histologischen Färbung* (*Contributions to the Theory and Practice of Histological Staining*), during which he discovered a new type of cell in blood. Ehrlich named his new discovery the mast cell (derived from the German word for an animal-fattening feed, *mast*) as he thought that the presence of many granules in the cytoplasm (made visible with the help of an alkaline dye) was a sign of good nourishment [17]. He also presented an entire spectrum of cell-staining techniques and chemistry of pigment dyes. Ehrlich continued to perfect his immunohistochemistry techniques at the Charité Hospital in Berlin where he used both alkaline and acidic dyes to create neutral-pH dyes for staining blood samples. These chemical dyes revealed different types of leukocytes that could be identified under the microscope based on the staining of their granules and nuclei. Nongranular lymphocytes, mono- and polynuclear leukocytes, neutrophilic and eosinophilic granulocytes, and mast cells, as well as some bloodborne pathogens, were distinguishable from each other. This laid the foundation for clinical diagnostics in haematology and pathology [16].

Ehrlich's innovation stemmed from his obsession with structural organic chemistry and selective cell staining with dyes for microscopic examinations. He was passionate about the idea that different chemicals could interact specifically with cells or proteins in pathological states and devised experiments for "in vivo staining" and therapeutic targeting. This led to the development of chemotherapy and the

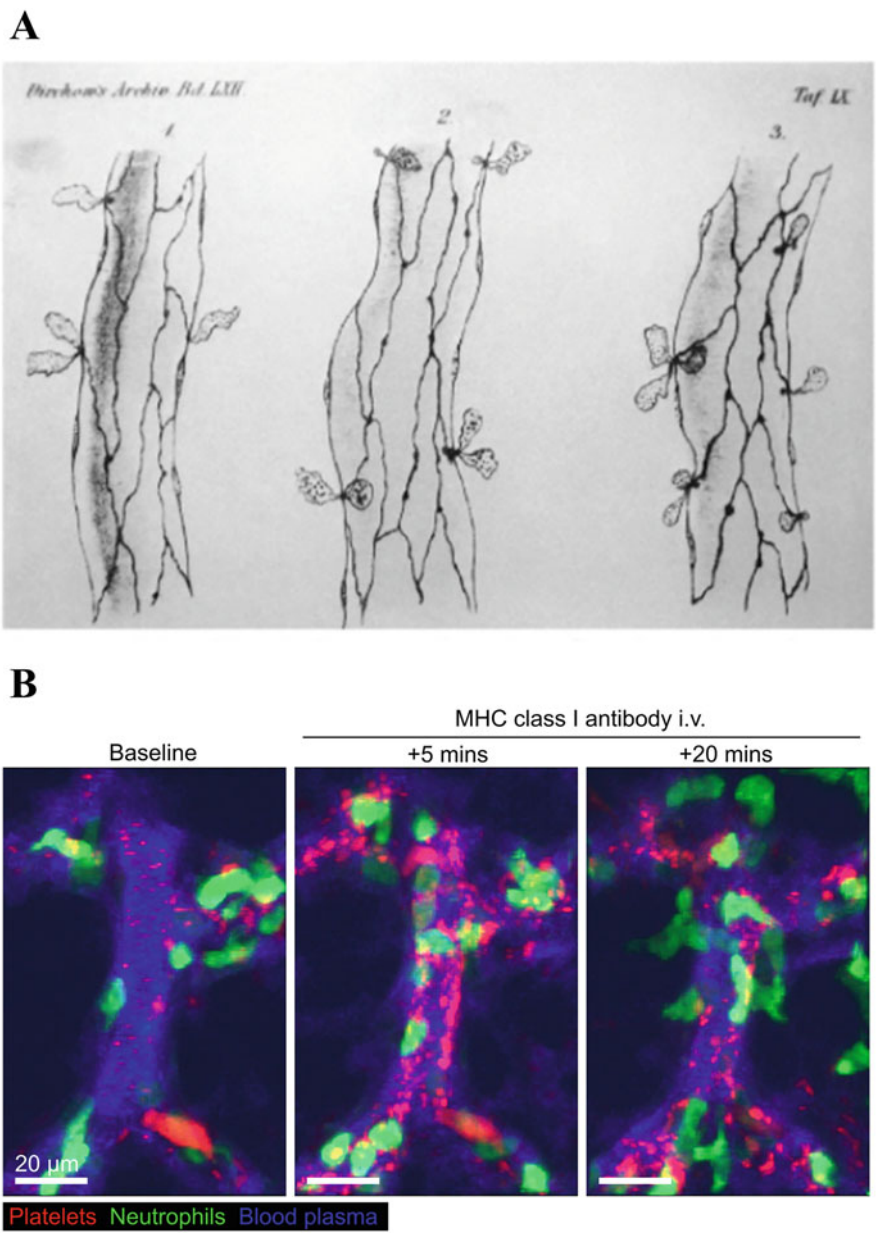

Fig. 2 Leukocytes exiting from the inflamed blood vessels. (**a**) Historical drawings of leukocyte transmigration at the blood vessels of a frog's tongue, mesentery, and bladder. From Arnold J., in *Archiv für Pathologische Anatomie und Physiologie und für Klinische Medicin*, Virchow R. (ed.) 1875, vol 62, page 487. The drawings are juxtaposed to (**b**) modern micrographs obtained on intravital microscopy. Reproduced with permission under CC-BY-4.0 licence, © 2020, Cleary et al. [14]

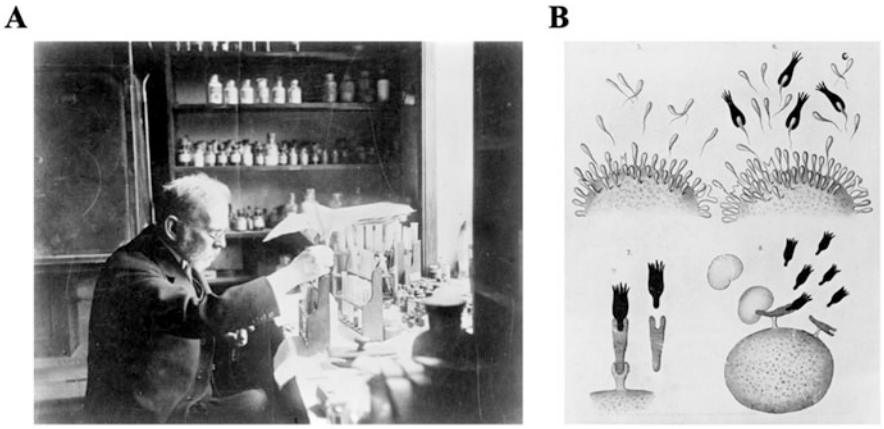

Fig. 3 Paul Ehrlich used chemistry to characterise leukocytes and tissues under the microscope, thereby establishing the "magic bullet" and "side-chain" theories. These influenced the development of molecular imaging techniques years later for theranostic applications. (**a**) Portrait of Paul Ehrlich performing immunohistochemistry in his laboratory. (**b**) Diagram on Ehrlich's side-chain theory illustrating the formation and effector functions of antibodies binding to antigens. Proceedings of the Royal Society of London (1856–1905). (**a**, **b**) reproduced with permission under CC-BY-4.0 licence. Credit: Wellcome Collection

establishment of the "magic bullet" theory [18]. In 1896, Ehrlich joined the Institute for Serum Research and Testing as its founding director and began work on antitoxins for diphtheria and their binding to antibodies in the blood. Ever an organic chemist and microscopist at heart, Ehrlich was fascinated by the idea of a specific immune reaction and its cellular and serological basis. He formulated thoughts on the "side-chain" theory and drew an analogy between antibody production and antigen recognition with how the side chains of chemical dyes are related to their colouring properties in cells or tissues visualised under the microscope [19]. By providing a theoretical and chemical basis for immunology, Ehrlich was jointly awarded the Nobel Prize for Physiology or Medicine in 1908 with Élie Metchnikoff, the "Father of Innate Immunity," who established the theory of cell-mediated immunity [19]. Interestingly, Ehrlich's early contributions to the "side-chain" theory of antibody-antigen interactions and the "magic bullet" theory of specific targeting in diseases also laid the foundation for research in molecular imaging and therapy (*theranostics*). Chemical agents based on labelled antibodies, peptides, and small molecules to target specific proteins expressed on abnormal cells or biological processes in pathological conditions such as cancer, infection, and inflammatory disorders have become important tools for radionuclide imaging and therapy [20]. With the advent of cryogenic electron microscopy (cryo-EM) in the 1970s, the natural structure of biological specimens can be preserved, embedded in their native environment, and protected from damage by electron beams using rapid cooling techniques [21]. 3D images at near-atomic resolution can now be obtained for studying the subcellular structures of viruses, macromolecules, receptor-ligand

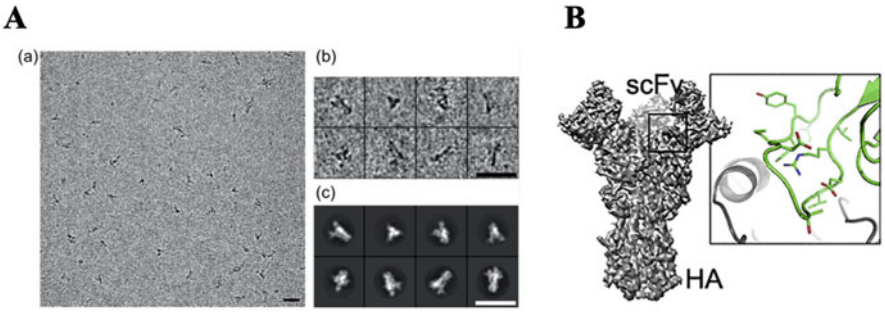

Fig. 4 Structure-based vaccine design using single-particle cryoEM as a tool to image the structure of the antibody-antigen complexes, e.g., influenza virus hemagglutinin (HA):single-chain variable domain fragment (scFv). (**a**) Cryomicrographs at different stages of image processing. (**b**) 3D surface rendering of the HA:scFv structure. Reproduced from Liu et al. [24] with permission, © 2017, Elsevier Ltd.

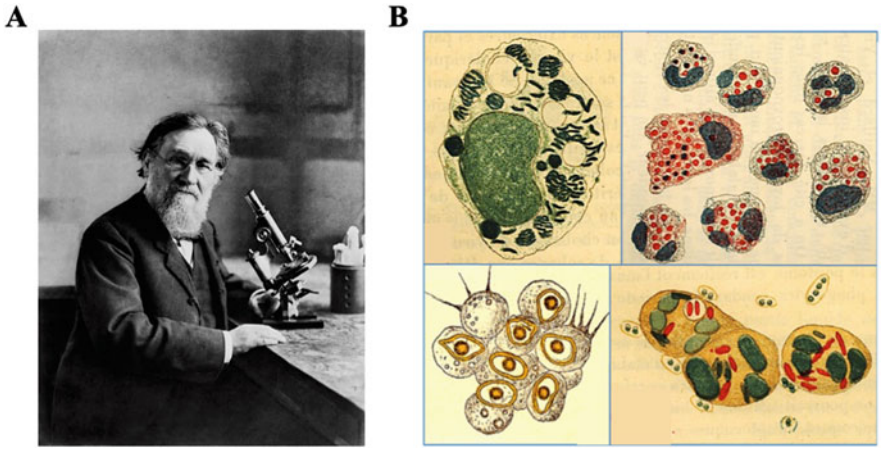

Fig. 5 Élie Metchnikoff was an enthusiastic microscopist and evolutionary embryologist who established the theory of "cell-mediated immunity." (**a**) Photograph: portrait of Élie Metchnikoff in his laboratory. Reproduced with permission under CC-BY-4.0 licence. Credit: Wellcome Collection. (**b**) Intricate drawings of phagocytosis by Metchnikoff (from top left to bottom right): a macrophage filled with *Vibrio cholerae*; leukocytes from a rabbit filled with tetanus spores; macrophages engulfing damaged erythrocytes; peritoneal fluid containing free streptococci and macrophages engulfing *Proteus bacilli*. From Metchnikoff, E. (1901) *L'immunité dans les maladies infectieuses.* Paris, Masson

interactions (e.g., the T cell receptor (TCR)-CD3 complex), as well as antibody-antigen interactions for structure-based drugs and vaccine design as shown in Fig. 4 [22, 23].

Élie Metchnikoff (Fig. 5a) would use microscopy to discover a major reason why leukocytes migrate out of inflamed blood vessels (Fig. 3). In 1882, Metchnikoff was on a working vacation with his family in Messina, Sicily, when he discovered the

process of phagocytosis in a starfish larva at the Mediterranean coast—a "Eureka!" moment while on holiday [25]. He recounted, "One day when the whole family had gone to a circus to see some extraordinary performing apes, I remained alone with my microscope, observing the life in the mobile cells of a transparent starfish larva, when a new thought suddenly flashed across my brain. It struck me that similar cells might serve in the defence of the organism against intruders. Feeling that there was in this something of surpassing interest, I felt so excited that I began striding up and down the room and even went to the seashore to collect my thoughts... I said to myself that, if my supposition was true, a splinter introduced into the body of a starfish larva, devoid of blood vessels or a nervous system, should soon be surrounded by mobile cells as is to be observed in a man who runs a splinter into his finger. This was no sooner said than done... There was a small garden to our dwelling, in which we had a few days previously organised a 'Christmas tree' for the children on a little tangerine tree; I fetched from it a few rose thorns and introduced them at once under the skin of some beautiful starfish larvae as transparent as water... I was too excited to sleep that night in the expectation of the results of my experiment, and very early the next morning I ascertained that it had finally succeeded... That experiment formed the basis of the phagocyte theory, to the development of which I devoted the next twenty-five years of my life" [25]. Metchnikoff named these mobile cells "phagocytes" from the Greek words *phagein* meaning "to eat" and *kytos* meaning "cell." In 1883, he published a key paper describing phagocytosis in frogs [26]. He explained how phagocytosis was important not just for host defense but also the elimination of degenerating or dying cells in the same host during metamorphosis from tadpoles to adult frogs: "The traits of the phagocyte have been retained most completely in the mesoderm where a large number of amoeboid cells occur to ingest the body's own dead or weak as well as foreign particles such as senescent red blood cells." This was one of the earliest descriptions of the function of our innate immune system in recognising self from nonself [27]. He described the engulfment of foreign particles and the morphological changes in the phagocytes he observed under the microscope (Fig. 5b) [28]: "These cells accumulate at the point of inflammation and devour the particles available to them. I have observed, for example, that star-shaped stromal cells feed on red blood cells, carmine and pigment particles. In cases where such cells ingest small numbers of foreign particles, they maintain their star-like shape with only some minor changes in the finest pseudopodia."

Metchnikoff combined microscopy and cell staining with neutral red and other dyes to evaluate the acidity of phagosomes, cell viability, and the fate of ingested microorganisms and foreign particles [29]. He took a comparative approach to infection and immunity, embracing natural history with experimentation in a wide range of model organisms from unicellular protozoa to transparent invertebrates, rodents, and even primates and humans [29]. Interpretations of microscopy experiments by Metchnikoff established the concept of cell-mediated immunity, while the microscopy studies of Ehrlich laid the foundation for humoral immunity [30]. Intravital microscopy has continued to expand our understanding of the immune system. It offers the opportunity for direct visualisation and characterisation of cellular

behaviour and the spatiotemporal dynamics of physiological processes within living organisms [31]. These include imaging biological events such as chemokine signalling and cell migration, the leukocyte adhesion cascade, endothelial transmigration, phagocytosis of foreign pathogens and damaged cells by macrophages and neutrophils, antigen presentation, and activation of effector T cells (Fig. 6) [32–34]. The ability to capture the cellular and molecular events of the immune system has improved our understanding on the biological mechanisms involved in health and diseases and equipped us with new knowledge in developing novel immunotherapies [35]. For applications of intravital microscopy to immunological problems and particularly inflammation, we direct the reader to the dedicated chapter *Imaging Inflammation by Intravital Microscopy* by De Donatis et al. in this book. Other advanced imaging techniques based on optical excitation and detection of biological events from a single-cell or subcellular level to whole-body scale are described in the chapters: *Spatial Transcriptomics in Inflammation: Dissecting the Immune Response in 3D in Complex Tissues* by Hu and *Whole-Body Chemiluminescence and Fluorescence Imaging of Inflammation* by Tseng and Peterson.

3 The Dawn of Radiology and Noninvasive Imaging of Inflammation

Metchnikoff and Ehrlich lived in a time when numerous giants of the scientific disciplines made outstanding contributions to medicine and influenced future research in imaging inflammation (Fig. 7). Most notably, the serendipitous discovery of X-rays in December 1895 by the German physicist Wilhelm Conrad Röntgen (1845–1923) marked the dawn of radiology and the emergence of noninvasive whole-body imaging techniques for disease diagnosis [36, 37]. While experimenting with the flow of electric current in a cathode ray tube, Röntgen observed a fluorescent effect on a small cardboard screen painted with barium platinocyanide when the tube was in operation. He reasoned that the fluorescence was caused by invisible rays originating from the cathode ray tube, which penetrated the opaque black paper wrapped around the tube. Further experiments were performed which confirmed that this unknown ray (thereby designated as “X”) could pass through most substances, including the soft tissues of the body, but was absorbed by dense materials such as bones and metallic objects [38]. Six weeks following his discovery, Röntgen took the first medical X-ray of the hand of his wife Anna Bertha. After seeing the ghostly image of the bones in her hand for the first time and her wedding ring “hovering” on one finger, she reportedly exclaimed, “I have seen my death.” For his discovery of X-rays and its potential use in medicine, Wilhelm Conrad Röntgen was awarded the Nobel Prize in Physics in 1901 [38]. Doctors began as early as January 1896 to use X-rays on patients for investigating skeletal abnormalities and subsequently to explore the lungs and other organs [39]. A simple chest X-ray and cross-sectional imaging using computed tomography (CT; pioneered by Sir Godfrey Hounsfield

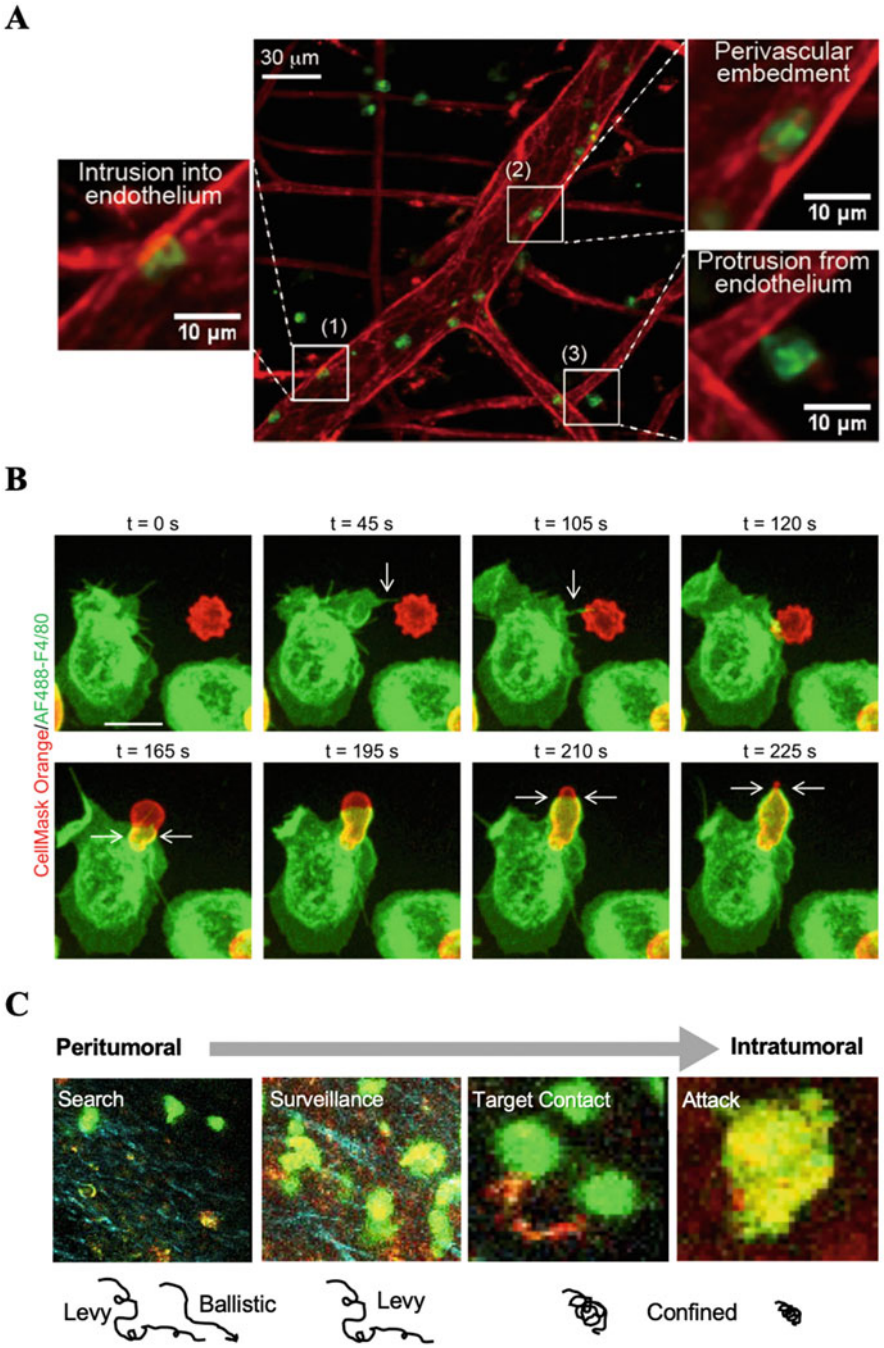

Fig. 6 Key concepts in immunology observed with intravital microscopy. (**a**) Neutrophil extravasation in tissues is a multistep event involving the adhesion of neutrophils in the blood to the vascular endothelium and eventual transmigration through the vessel wall to reach the site of infection or inflammation. Reproduced from [32] with permission. © 2019, Hyun et al.

(1919–2004) and Allan Cormack (1924–1998)) have become routine tests for diagnosing tissue structural abnormalities in many inflammatory conditions such as pneumonia, bronchitis, and even acute respiratory distress syndrome (ARDS) associated with COVID-19 infection [40, 41].

The discovery of spontaneous radioactivity by the French physicist Henri Becquerel (1852–1908) is another famous example of serendipity in science [43]. In early 1896, after a wave of excitement following Röntgen's discovery of X-rays, Becquerel was investigating the relationship between the absorption of light and phosphorescence in uranium compounds when he suspected that his luminescent materials might emit some of these mysterious X-rays. He began his own study of the X-rays by simulating Röntgen's experiments and accidentally discovered the spontaneous emission of radiation from the uranium compounds he kept in a dark drawer with a photographic plate [44]. A period of intense research on radioactivity followed, including the discovery of additional radioactive elements polonium and radium by the French couple Pierre Curie (1859–1906) and Marie Skłodowska Curie (1867–1934) [45]. Marie Curie ingeniously used a piezoelectric quartz electrometer developed by Pierre Curie and his brother Jacques Curie (1855–1941) and attached it to an ionisation chamber to search for elements that emit ionising radiation. This enabled the quantification of radioactivity and discovery of medically useful radioactive elements such as radium. In 1903, Henri Becquerel and Marie and Pierre Curie were jointly awarded the Nobel Prize in Physics for their research on radioactivity. For her discovery and isolation of radium and polonium, Marie Curie was awarded the Nobel Prize in Chemistry in 1911. Many decades later, further developments by others in the field led to the discovery of gamma-emitting and positron-emitting radioisotopes, forming the basis of radionuclide imaging and nuclear medicine [46].

Gallium-67 (^{67}Ga)-labelled citrate has been used for scintigraphy and single-photon emission computed tomography (SPECT) of infections and inflammatory disorders for over 30 years. Gallium functions as an iron analogue in vivo by binding to transferrin and extravasates within inflammatory sites due to increased blood flow and vascular permeability. However, gallium-67 has a long physical half-life (78 hours) and exhibits nonspecific uptake in tissues such as the liver, nasopharynx, and lacrimal and salivary glands [47, 48]. This imaging method has now been superseded by techniques such as white blood cell (WBC) scintigraphy or SPECT and fluorine-18 fluorodeoxyglucose ([^{18}F]FDG) positron emission tomography

Fig. 6 (continued) (CC-BY-4.0 license). (**b**) Snapshots from a time-lapse video showing a mouse macrophage capturing a mouse immunoglobulin G (IgG)-opsonised human erythrocyte via a filopodium. This was followed by the formation of a phagocytic cup and engulfment of the erythrocyte. Images reproduced from Horsthemke et al. [33] with permission. (**c**) Antigen-specific T cells demonstrated distinct behaviours during tumour infiltration. The interactions between T cells and their target cancer cells during antigen presentation and T cell cytotoxic attack on the cancer cells were captured in living mice on intravital microscopy. Images reproduced with permission under CC-BY-4.0 licence, © 2020, Lau et al. [34]

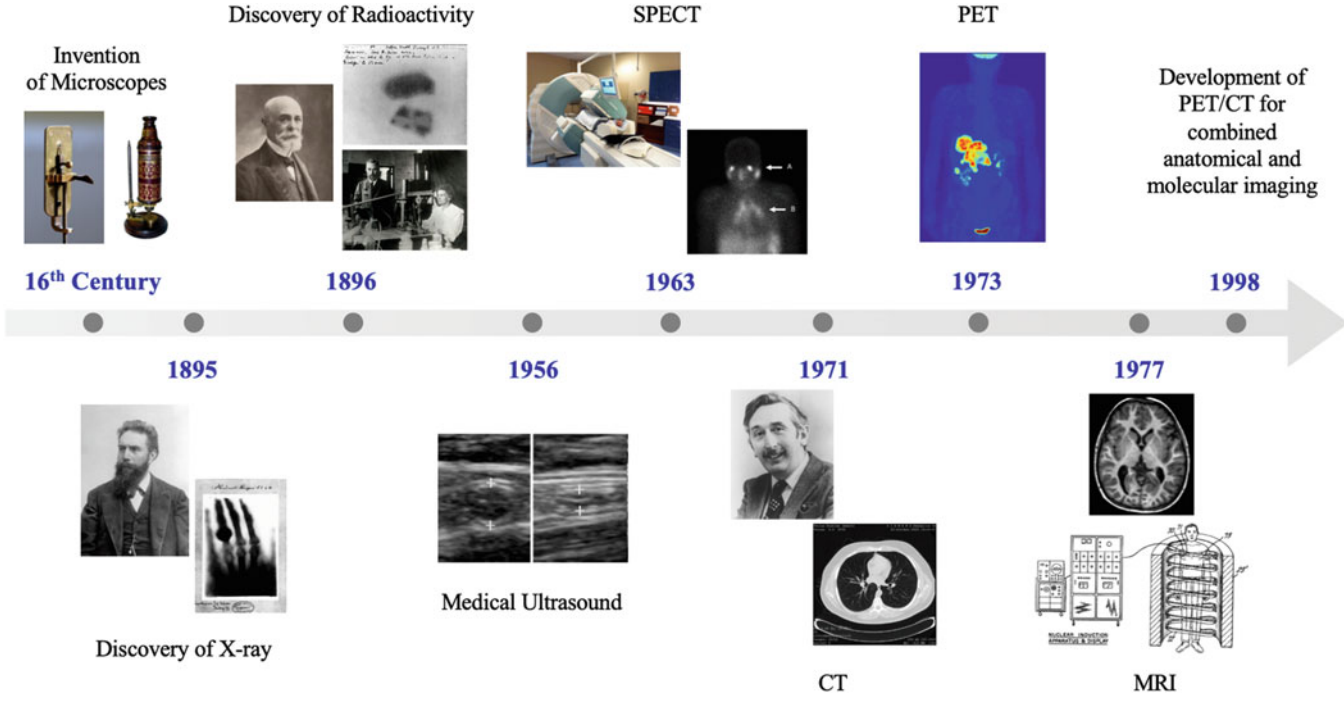

Fig. 7 Historical timeline of the invention of biomedical imaging tools. Illustration by the author (D. Lau) created from Wikimedia public domain images unless otherwise indicated. Top line, left to right: replica of a van Leeuwenhoek microscope, image by Jeroen Rouwkema (CC BY-SA 3.0 license); Hooke microscope, Billings Microscope Collection, National Museum; portrait of Henri Becquerel by Paul Nadar, ca. 1905; photographic plate by H. Becquerel showing effects of exposure to radioactivity; portrait of Pierre Curie and Marie Skłodowska-Curie; SPECT/CT scanner, image by Wikimedia user Ytrottier (CC BY-SA 3.0 license); gallium-67 scan of a patient with sarcoidosis, reproduced from [42] under CC BY-SA 4.0 license, © 2014, Allard et al.; maximum intensity projection of an [^{18}F]FDG-PET/CT scan of a patient with metastatic colorectal cancer, image by Jens Langner. Bottom line, left to right: portrait of Wilhelm Röntgen; X-ray of Anna Röntgen's hand by W. Röntgen; ultrasound image of the appendix, without and with compression, Mikael Häggström; portrait of Godfrey Hounsfield ca. 1975; frame from a chest CT scan, image by Wikimedia user Ptrump16 (CC BY-SA 4.0 license); schematic drawing of a prototypical MRI scanner, 1972, Raymond Damadian, (US government Patent number 3789832)

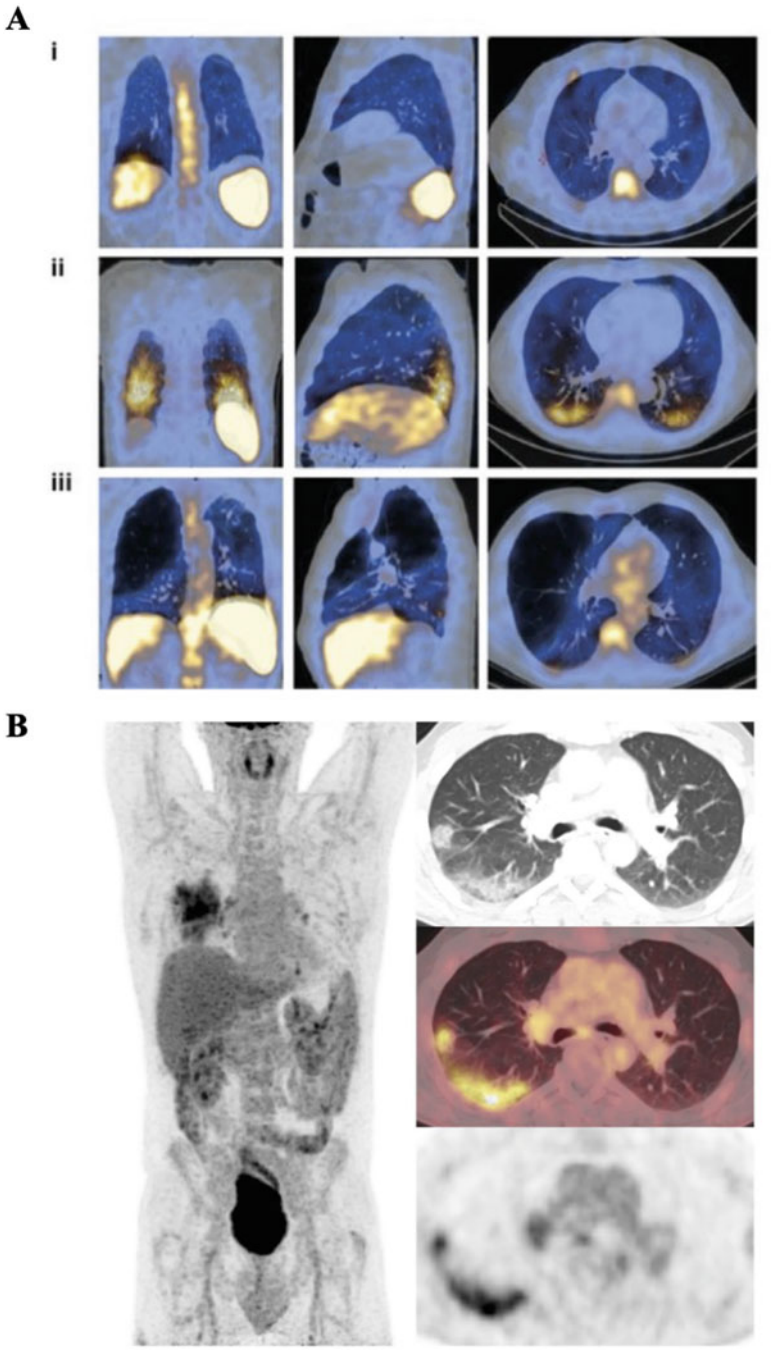

Fig. 8 White blood cell scintigraphy or SPECT/CT and [^{18}F]FDG PET/CT are routine clinical imaging techniques used for imaging different biological processes in infections and inflammatory disorders. (**a**) SPECT imaging of [^{99m}Tc]Tc-HMPAO-radiolabelled autologous neutrophils has been used in experimental medicine studies for studying chronic obstructive pulmonary disease

(PET) as shown in Fig. 8 [49, 50]. WBC scintigraphy is a gold standard technique introduced in 1976 for routine clinical use in detecting inflammation and unknown sites of infection, e.g., osteomyelitis and vascular graft infections [49]. The technique involves the ex vivo radiolabelling of autologous leukocytes (primarily neutrophils) using lipophilic, cell-permeable radiopharmaceuticals such as technetium-99m (^{99m}Tc) hexamethylpropyleneamine oxime (HMPAO) and indium-111 (^{111}In) oxine to image the migration kinetics of the radiolabelled cells and infiltration at the inflamed tissues following reinjection [51]. In recent years, SPECT imaging of radiolabelled autologous neutrophils has also been used in experimental medicine studies to investigate changes in neutrophil migratory behaviour, tissues, and systemic biodistribution in lung inflammatory disorders such as ARDS and chronic obstructive pulmonary disease (COPD) [52, 53]. On the other hand, [^{18}F]FDG is a radiolabelled glucose analogue that can be taken up by glucose transporters and remain trapped in cells following phosphorylation by hexokinase. Leukocytes such as neutrophils, monocytes, and macrophages exhibit higher expression of glucose transporters such as GLUT1 and rapidly increase their glucose consumption upon activation [54]. [^{18}F]FDG PET has been used for detecting metabolic inflammatory foci in nonmalignant conditions such as tuberculosis and inflammatory bowel disease [55, 56]. However, the use of [^{18}F]FDG PET for monitoring infection or inflammatory processes in the presence of malignancy can often complicate diagnosis as both cancer cells and leukocytes are metabolically active cell types [50]. Thus, special precautions and clinical triage should be made when interpreting radiological scans in the presence of both diseases [57]. In recent years, novel radiopharmaceuticals targeting the expression of immune-related biomarkers such as CD8, granzyme B, and PD-1 have been developed for immuno-oncology applications [58–60]. These methods enable the detection and characterisation of specific tissue-resident leukocyte subtypes, as well as the detection of markers of leukocyte activation or immunosuppression within inflamed tissues as potential alternative approaches to biopsy. PET and SPECT imaging of inflammation are further detailed by Keeling and Man in the chapter *Nuclear Imaging of Inflammation* of this book.

The discovery of the piezoelectric properties of quartz deformation by Pierre and Jacques Curie also laid the foundation for the development of ultrasound imaging. Ultrasound was first used in 1917 during World War I by the physicist Paul Langevin (1872–1946) for the detection of submarines [61]. The technology was subsequently adapted for medical ultrasound in 1956 by Ian Donald (1910–1987),

Fig. 8 (continued) (COPD). Shown over here are the 45 min thoracic SPECT/CT scans of (i) a saline-challenged healthy volunteer, (ii) a healthy volunteer challenged with lipopolysaccharide, and (iii) a patient with COPD, showing differences in neutrophil migration and tissue infiltration in the inflamed lungs. Images reproduced with permission under CC-BY-4.0 licence, © 2019, Tregay et al. [53]. (**b**) [^{18}F]FDG PET of a cancer patient with COVID-19, showing incidental findings on follow-up scans of a new hypermetabolic area in the right upper and lower lung. Ground glass opacities in the periphery/subpleural regions of the lungs were concurrently seen on CT. Images reproduced with permission from Czernin et al. [57] under CC-BY-4.0 licence, © 2020, Society of Nuclear Medicine and Molecular Imaging

which uses handheld ultrasonic probes or transducers comprising of piezoelectric crystals and alternating current electrodes to transmit and receive high-frequency sound waves through tissues. The reflected echoes from tissues result in deformation of the piezoelectric components and interconversion of the mechanical energy into electrical pulses. Images reflecting the stiffness and density of the tissues are produced based on the depth and speed of sound waves propagating through tissues [62]. Ultrasound is a relatively low-cost technique suitable for imaging soft tissues and real-time monitoring of blood flow using Doppler imaging. Ultrasound is particularly useful for road-mapping and guiding needle intervention. It is commonly used for image-guided tumour biopsy in cancer diagnosis [63], and there is increasing interest in using ultrasound for image guidance during intratumoral delivery of immunotherapies into deeper and non-palpable tumours [64], as well as focused ultrasound for immunomodulation of the tumour microenvironment [65]. Ultrasound has been used for visualising needle puncture and draining of abscesses or postoperative collections from infected tissues [66]. It enables the identification of adjacent anatomical structures and helps determine the best tract and safest route for intervention [67]. As ultrasound does not involve the use of ionising radiation and the scanner is usually mobile and can be made into portable handheld versions, it is used in settings such as paediatrics, critical care, and emergency medicine for point-of-care bedside imaging of infections in the lungs and pleural cavity, soft tissues, and musculoskeletal regions and the monitoring of interventional procedures [68–70]. The use of ultrasound imaging in inflammation is further detailed in the dedicated chapter of this book *Ultrasound Imaging in Inflammation Research* by Muller et al. A related and more recent technique is photoacoustic imaging, which is based on the detection of ultrasound waves generated by optical excitation of tissues and contrast agents; see the chapter *Photoacoustic Imaging in Inflammation Research* by Chen and Xie et al. in this book.

In the twentieth century, the discovery of nuclear magnetic resonance (NMR) in biomolecules and developments in imaging physics and instrumentation for spatial encoding of NMR signals using magnetic field gradients by the British and American trio Sir Peter Mansfield (1933–2017), Paul Lauterbur (1929–2007), and Raymond Damadian (born 1936) led to the introduction of magnetic resonance imaging (MRI) [71]. MRI is now a clinically available tool in hospitals for disease diagnosis and treatment monitoring. Images with excellent soft tissue contrast and high spatial resolution can be obtained without the use of ionising radiation, together with physiological measurements of biological processes such as blood flow, edema, and cellular changes associated with inflammation. MRI is based on the principle of NMR, in which certain atomic nuclei such as protons (^{1}H) absorb and re-emit radiofrequency energy when placed in an external magnetic field [72]. Tissue contrast obtained on MRI is based on the spin-lattice relaxation (T_1) and spin-spin relaxation (T_2) times, which are dependent on the chemical structures of the molecules imaged and the local chemical environment within the tissues [73]. Several MRI and MR spectroscopic techniques have been developed over the years for imaging microstructural, functional, and molecular changes in infection and