ANNUAL REVIEWS

Annual Review of Immunology

Spatiotemporal Adaptations of Macrophage and Dendritic Cell Development and Function

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Annu. Rev. Immunol. 2022. 40:525–57

First published as a Review in Advance on February 7, 2022

The *Annual Review of Immunology* is online at immunol.annualreviews.org

https://doi.org/10.1146/annurev-immunol-101320-031931

Keywords

antigen presentation, sepsis, inflammation, immunosuppression, immune training, COVID-19

Abstract

Macrophages and conventional dendritic cells (cDCs) are distributed throughout the body, maintaining tissue homeostasis and tolerance to self and orchestrating innate and adaptive immunity against infection and cancer. As they complement each other, it is important to understand how they cooperate and the mechanisms that integrate their functions. Both are exposed to commensal microbes, pathogens, and other environmental challenges that differ widely among anatomical locations and over time. To adjust to these varying conditions, macrophages and cDCs acquire spatiotemporal adaptations (STAs) at different stages of their life cycle that determine how they respond to infection. The STAs acquired in response to previous infections can result in increased responsiveness to infection, termed training, or in reduced responses, termed paralysis, which in extreme cases can cause immunosuppression. Understanding the developmental stage and location where macrophages and cDCs acquire their STAs, and the molecular and cellular players involved in their induction, may afford opportunities to harness their beneficial outcomes and avoid or reverse their deleterious effects. Here we review our current understanding of macrophage and cDC development, life cycle, function, and STA acquisition before, during, and after infection.

We propose a unified framework to explain how these two cell types adjust their activities to changing conditions over space and time to coordinate their immunosurveillance functions.

1. INTRODUCTION

Macrophages and conventional dendritic cells (cDCs) provide the first line of immunosurveillance. Both types of cells are present throughout the body, strategically positioned at sites of pathogen entry or dissemination. They are endowed with one or more of the major forms of endocytosis required to gain information about the health of the extracellular environment and the presence of pathogens: phagocytosis, macropinocytosis, micropinocytosis, and receptor-mediated endocytosis (1). Both cell types have the capacity to detect pathogens (2) or tissue damage (3) and perform activities associated with the innate and adaptive arms of the immune system to sterilize tissues and restore homeostasis (4). They achieve this by secreting cytokines that cause inflammation and activation of other innate cells [e.g., natural killer (NK) cells] and interacting with T cells via antigen presentation to initiate or regulate adaptive immunity (5–8). Their activities are not additive nor contradictory, but cooperative. Yet, studies on the function of macrophages and cDCs are generally performed by two different sets of scientists, and their findings are rarely published in the same papers. This is because the ontogeny and functional specializations of the two cell types are sufficiently different and complex to require separate experimental models, technical approaches, and academic backgrounds to undertake their study. Macrophages not only are involved in immunity (9) but also carry out tissue maintenance (10, 11), whereas cDCs are biased toward immune functions (12, 13). The immunosurveillance function of macrophages is archetypically innate, biased toward noninflammatory elimination of microbes when the level of infection is low but initiation of inflammation when pathogens spread. The immunosurveillance function of cDCs is to present antigens to T cells in secondary lymphoid organs to induce tolerance to self-components but initiate adaptive immunity upon encounter of pathogens or tumors. Protection against harmful pathogens must at the same time avoid reactions against beneficial or innocuous microbiota, which are tolerated by tonic induction of homeostatic immunity that involves every component of the immune system, including macrophages and cDCs (14). Thus, both macrophages and cDCs have to maintain an equilibrium between avoiding immune reactions that may be unnecessary and even harmful and inducing protective immunity, according to the nature of the challenge (15). A central tenet in this review is that to maintain this equilibrium, the immune responsiveness of macrophages and cDCs must be tuned to the specific conditions of different anatomical locations and continually adjusted to changes in pathogen type or abundance, frequency of infection, and other environmental challenges.

The advances made by the macrophage and cDC fields over the last two decades are dramatic, and it is understandable that cDC researchers may find it difficult to keep abreast with progress in the macrophage field, and vice versa. Our intention with this review is to bridge the two areas of research to the largest extent possible, presenting an overview of the current state of the macrophage and cDC fields and identifying major knowledge gaps and areas for development. We unintentionally fail to give proper credit to all the scientists who have contributed to elucidating the life cycle and function of macrophages and cDCs, as it is impossible to discuss all relevant work in a single review, and we apologize for our shortcomings.

Over the years, several cell types have been defined as dendritic cells or macrophages according to changing phenotypic and functional definitions (16), but we follow the recommendation to use ontogeny as the basis for cellular classifications (12, 17) (see the sidebar titled Terms and Definitions). Our definition of cDCs therefore does not include plasmacytoid DCs (pDCs), which

TERMS AND DEFINITIONS

The following are definitions of commonly used terms in this review.

Conventional dendritic cells (cDCs): Short-lived cells that differentiate from bone marrow precursors. Their main functions are to capture, process, and present antigens to, and activate, naive CD4 and CD8 T cells.

Plasmacytoid dendritic cells (pDCs): While ontogenically related to cDCs, pDCs have different life cycles and are functionally distinct from cDCs, being endowed with a high capacity to release type I interferons but limited capacity for antigen capture, processing, and presentation and naive T cell activation.

Monocyte-derived cells: Cells derived from bone marrow precursors that differentiate into macrophages or DC-like cells at sites of infection and/or inflammation. The categorization of these cells as DCs is controversial. In this review they are referred to as I-MACs.

Resident macrophages (R-MACs): Macrophages derived from yolk sac precursor cells, fetal liver precursor cells, or bone marrow monocytes, in the absence of overt inflammation and microbes other than the microbiota. R-MACs self-renew in their tissue of residence. They are generally anti- or hypoinflammatory.

Inflammatory macrophages (I-MACs): Macrophages derived from bone marrow precursors and recruited to sites of infection and/or inflammation. Once generated, I-MACs can stay in the tissue and become a self-renewing population. They are generally proinflammatory.

Efferocytosis: Phagocytosis of dead or dying cells.

Endogenous and exogenous antigens: Proteins synthesized and not synthesized, respectively, by an antigen-presenting cell. The term endogenous is often used as synonymous with cytosolic, but the words endogenous and exogenous define origin, not location. All proteins synthesized by a cell are endogenous, regardless of their subcellular localization (cytosol, plasma membrane, lumen of intracellular compartments, etc.). Conversely, any protein not synthesized by a cell is exogenous.

MHC-I- and MHC-II-presented antigens: The most rigorous way to differentiate these two types of antigens is based on the location where they are processed into peptides. MHC-I-presented antigens are degraded in the cytosol, and MHC-II-presented antigens are degraded in the lumen of endosomal compartments. The terms endogenous and exogenous should be avoided in this context, as some cells can present exogenous antigens via MHC-I (cross-presentation) and all cells present both endogenous and exogenous antigens via MHC-II.

Cross-presentation: Presentation of exogenous antigens via MHC-I molecules.

Systemic Inflammatory Response Syndrome (SIRS): A pathology caused by excessive production of inflammatory cytokines that leads to organ damage. It can be initiated by an infection (sepsis) or excessive sterile inflammation, for example, severe trauma, stroke, or burns. The clinical presentation of SIRS includes fever, tachycardia, polypnea, and hyperleukocytosis.

Sepsis: A form of SIRS that has at its origin a confirmed or suspected infection and is complicated by acute organ failure. The infection does not need to be located in the blood (septicemia), as is often thought. It can affect any organ.

Spatiotemporal adaptation (STA): The term as used in this review refers to a collection of molecular changes experienced by developing macrophages and cDCs, including gene transcription, epigenetic regulation, protein synthesis and turnover, metabolism, etc., under the influence of their local environment. These changes fine-tune

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the immunological properties of the terminally developed macrophages and cDCs to the specific conditions of the tissue where they will carry out their functions.

Training: A process by which an STA makes a cell more immunologically responsive (e.g., prone to secrete more cytokines or to undergo activation in response to a lower stimulation threshold) than an equivalent cell type that has not been exposed to the stimuli that induced training. Such stimuli consist of pathogen-associated compounds (e.g., bacterial lipopolysaccharide) or secondary signals released during infection.

Tolerance: The term as used in this review refers to the immunologically less responsive state (e.g., less capable of phagocytosing bacteria) of a cell due to an STA compared to an equivalent cell type that has not been exposed to the stimuli that induced tolerance. Such stimuli consist of secondary signals released during infection or maintained locally at sites of prior infection.

Paralysis: Profound unresponsiveness induced in macrophages, cDCs, and other cell types as an extreme form of tolerogenic STA. Paralysis is a major contributor to long-term immunosuppression in individuals who recover from SIRS.

are ontogenically and functionally distinct (18). Likewise, we do not refer to monocyte-derived DCs, because all cells that develop from monocyte precursors are now considered ontogenically distinct, most likely part of the macrophage family but in any case separate from the cDC lineage (10, 12, 17, 19, 20). For simplicity, here we classify monocyte-derived cells as macrophages, albeit with the understanding that in the future these two may be considered separate cell lineages. Except where indicated, the term macrophage refers to all cell types that, although also known by their individual names (microglia, Kupfer cells, etc.), are components of the macrophage system (9, 10). Ironically, this includes Langerhans cells, long considered the archetypical cDCs (21–23) but now classified as a type of macrophage (10, 12, 17, 24–26). These cell type reassignments are illustrative of the magnitude of changes that have occurred in this area of immunology in just one decade.

2. MACROPHAGE AND cDC DEVELOPMENT AND FUNCTION IN STEADY-STATE MICE

2.1. Advantages and Limitations of the Specific-Pathogen-Free Mouse Model

Most of what we know about macrophage and cDC development has been learned from studies of mice housed in specific-pathogen-free (SPF) animal facilities. The term SPF implies that the only microorganisms encountered by the animals throughout their life span are commensal flora and pathogens that are ignored (they are assumed to be present in the facility but are not measured) or are too difficult to eliminate (27). There is no universal criterion to define an animal facility as SPF (as opposed to one where there has been an infection outbreak by an undesirable pathogen), so SPF mice in different locations may be exposed to different classes and/or levels of pathogen infection. Furthermore, commensal flora also varies among facilities (28, 29). Nevertheless, it is accepted that all SPF mice are exposed to a narrow variety of microorganisms, have a commensal flora that is less diverse than those found in wild or even pet-shop mice (30, 31), and are free from overt infections their entire life. These conditions are often referred to as steady state. The advantage of using SPF mice as a model to describe cellular ontogeny and function is that results from different laboratories are more comparable. The disadvantage is that SPF facilities do not recapitulate the microbe-rich environment where the mammalian immune system evolved.

Exposure to microbes affects the development of the cells of the immune system and their functional properties (32–34). SPF (and, presumably, wild) mice are born with an underdeveloped immune system that resembles that of human newborns, but whereas the immune system of adult wild mice or humans matures, the immune system of SPF mice maintains much of its original character (32). This difference affects the immune response against viruses, bacteria, protozoans, and tumors (30, 32; reviewed in 29, 35). Nevertheless, the SPF mouse has enabled detailed mapping of the development of macrophages and cDCs and will remain the preferred experimental model for immunology research for the foreseeable future. It provides a reproducible benchmark to characterize the function of macrophages and cDCs in the steady state and upon the introduction of perturbations caused by exposure to pathogens or environmental insults.

2.2. Development, Life Cycle, and Function of the Macrophage Lineage in Steady-State Mice

Macrophages derive from three main sources of precursor cells: the yolk sac, fetal liver, and bone marrow (**Figure 1**). Macrophages derive from the first two sources before or soon after birth, with their relative proportion varying among tissues, and from the third thereafter (10) (**Table 1**).

2.2.1. Macrophages develop from embryonic precursors before birth. Neither yolk sac–derived macrophages nor fetal liver–derived macrophages are homogeneous throughout the body. The precursors display considerable plasticity, and during their differentiation into macrophages in the skin, lung, gut, brain, etc., they respond to local cues that collectively create a unique niche. The cues in each niche determine macrophage abundance, positioning within the tissue, life span, and function (11, 36–38). The developing macrophages thus acquire unique transcriptomic, phenotypic, metabolic, and functional profiles specific for their tissue of residence (39–52) and adopt organ-specific identities (reviewed in 11, 36, 37). The macrophages thus become an inextricable component of the organ where they develop, interacting with other cells within the tissues and influencing each other (10). We refer henceforth to the collection of adaptations (phenotypic, epigenetic, transcriptomic, metabolic, etc.) acquired by macrophages (and cDCs) during their differentiation in tissues as spatiotemporal adaptations (STAs).

Embryonic macrophages can potentially live throughout the entire life span of the animal. In practice, small numbers are continually lost upon encounter of different insults and at variable rates depending on their anatomical location, but they are replenished by division of other macrophages in situ. As a rule, they do not leave the site where they develop. The inevitable exception is Langerhans cells, which migrate via lymph to skin-draining lymph nodes (21–23). This behavior is considered an attribute of cDCs and is one of the reasons why Langerhans cells resist classification despite their clear ontogenic inclusion within the macrophage lineage (17, 24; reviewed in 10, 12, 25). Indeed, replacement of migratory Langerhans cells occurs within the epidermis by self-renewal, an unequivocal macrophage property (24). A recent report may have reconciled these contradictory observations by showing that Langerhans cells in fact do not migrate out of the epidermis. The migratory cells that were described as Langerhans cells in previous studies corresponded to a monocyte-derived cell population of the dermis that shares phenotypic features with bona fide Langerhans cells and does migrate to the lymph nodes (26).

2.2.2. Bone marrow monocytes become resident macrophages after birth. After birth, $CCR2^+$ monocytes produced in the bone marrow enter circulation and can infiltrate tissues and become self-renewing macrophages that coexist with those derived from embryonic precursors (10, 11, 36, 37, 53). Each tissue contains a limited "space" for macrophages, so monocytes can only

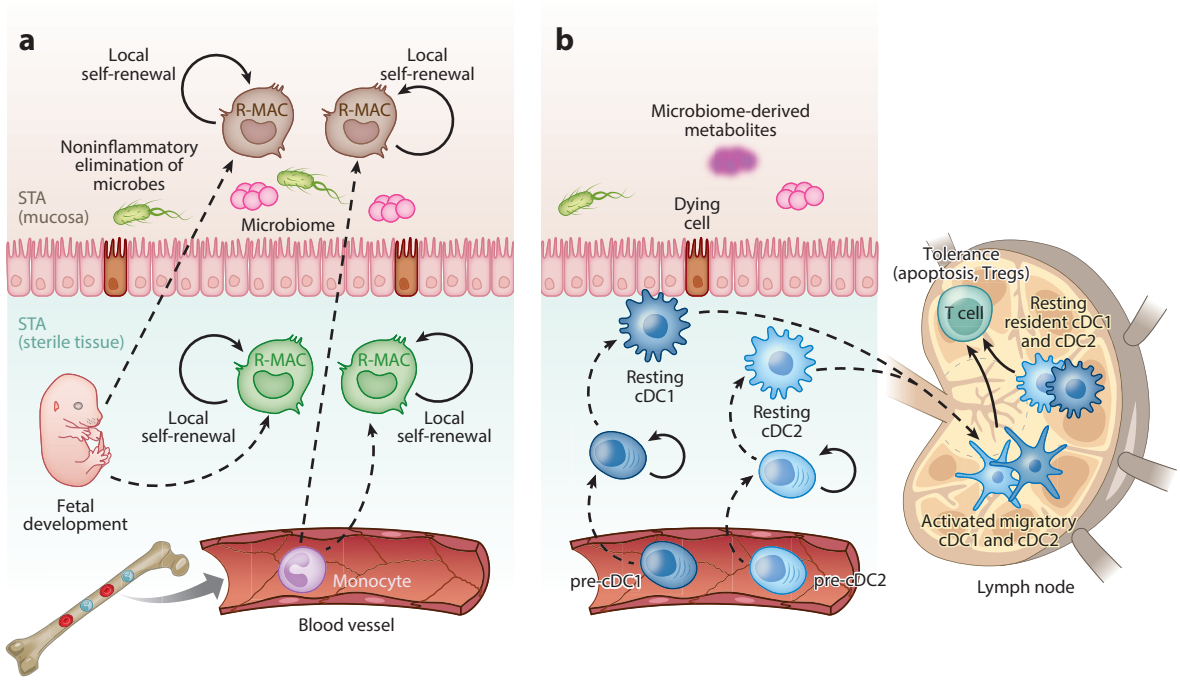

Figure 1

Macrophage and cDC life cycle in the steady state. (*a*) At the time of birth, all macrophages are derived from fetal precursors. These R-MACs reside in the tissue where they underwent final differentiation from earlier precursors, are long-lived, and self-renew. The R-MACs acquire STAs specific to their tissue of residence, shown here for a mucosal surface where microbes abound (*top*) and for sterile internal organs (*bottom*). After birth, monocytes derived from the bone marrow can enter tissues and also become R-MACs, replacing their fetal counterparts, and acquire the same STAs as their predecessors. The primary immunosurveillance role of R-MACs is the noninflammatory ingestion and elimination of microbes, unless infection reaches a level that triggers R-MAC-mediated inflammation (**Figure 2**). (*b*) Two types of cDCs, cDC1s and cDC2s, develop from pre-cDCs that leave the bone marrow and disseminate via blood to all tissues, where they undergo a few rounds of proliferation and final differentiation. New cDCs are in a resting state dedicated to patrolling and endocytosing material from their environment, in both the secondary lymphoid organs (resident cDCs) and nonlymphoid tissues (migratory cDCs). Of the two cDC types, only cDC1s can efferocytose dead cells (*brown*). The migratory cDCs spontaneously traffic via lymph to the local lymph node and in the process undergo a series of phenotypic and functional changes known as activation. The resident cDCs remain in a resting state throughout their lifespan. Naive T cells that recognize self-antigens presented by migratory activated cDCs or resident resting cDCs die, become unresponsive, or differentiate into Tregs, processes known as peripheral tolerance. Both migratory and resident cDCs die within lymphoid organs. As cDCs are short-lived, the cDC network is continuously renewed by newly arriving, bone marrow–derived, pre-cDCs. Abbreviations: cDC1, type 1 conventional dendritic cell; cDC2, type 2 conventional dendritic cell; R-MAC, resident macrophage; STA, spatiotemporal adaptation; Treg, regulatory T cell.

colonize and become macrophages in places left open by the temporal absence of their embryonic counterparts in the tissue niche. It is not entirely clear what determines the rate of monocyte recruitment to a particular organ in SPF mice, but the driving force is most likely the attrition of embryonic macrophages, which is in turn dependent on exposure to microbiota, pathogens, mechanical forces, environmental insults, or other sources of cellular stress. If the rate of embryonic macrophage losses surpasses the capacity of the remaining macrophages for local self-renewal, monocytes will occupy the available niche. Bone marrow–derived macrophages become the major population in the gut soon after weaning (54, 55). They are also abundant in the dermis (56), but the rate of their recruitment to other organs is more tenuous or negligible (57–60).

Table 1 Characteristics of murine macrophages and conventional dendritic cells

	Origin and renewal	Functional properties	Plasma membrane markers	Transcription factors
R-MACs	Before or soon after birth: embryonic precursors from the yolk sac or fetal liver After birth: circulating CCR2+ monocytes from bone marrow, in the absence of overt inflammation, replacing embryonically derived R-MACs Undergo local adaptation to tissue of residence Local self-renewal Slow turnover	Noninflammatory elimination of microbes Cloaking of tissue microlesions to limit inflammatory damage Tolerance Secretion of proinflammatory cytokines to recruit I-MACs, neutrophils, and other immune cells when infection cannot be controlled by R-MACs alone	$CD11b^{low}$ $CD11c^{+}$ $CD64^{+}$ $CD172a^{+}$ $CD206^{+}$ $CX3CR1^{-}$ $F4/80^{+}$ $Ly6C^{-}$ MHC-II^{-} Siglec-F^{+}	PU.1 c-MAF MAFB ZEB2 Tissue specific: PPARγ (alveoli), ID3 (liver), CEBPB (peritoneum)
I-MACs	Circulating $CCR2^{+}$ monocytes from bone marrow recruited to inflammation sites Replace and/or coexist with R-MACs Undergo local adaptation to inflamed tissue Local self-renewal Lifespan dependent on extent and duration of inflammation	Induction of inflammation Engulfment and destruction of pathogens Secretion of antimicrobial compounds Antigen presentation at the site of infection Profibrotic Tissue repair	$CD11b^{+}$ $CD11c^{low}$ $CD64^{+}$ $CD172a^{+}$ $F4/80^{+}$ $Ly6C^{+}$ MHC-II^{+} $CX3CR1^{+}$ $TREM2^{+}$	EAR2 MAFB KLF4
cDC1s	Local differentiation in all tissues from bone marrow precursors (pre-cDC1s) Rapid turnover Migratory (generated in nonlymphoid tissue): migrate to local lymph node via lymph and undergo nonimmunogenic (steady-state) or immunogenic (upon pathogen encounter) activation before dying Resident (generated in lymphoid organs): die in lymphoid organs without undergoing activation (in steady state) or after activation (upon pathogen encounter)	Survey local tissue and constitutively engulf extracellular material using multiple mechanisms of endocytosis (phagocytosis, macropinocytosis, and receptor-mediated endocytosis) Efferocytosis MHC-II presentation Cross-presentation Priming of naive CD4 and CD8 T cells IL-12 and IFN-III secretion Transfer of MHC-II and other plasma membrane receptors to marginal zone B cells via trogocytosis	$CD11c^{+}$ $CD24^{+}$ $CD205^{+}$ $CD207^{+}$ $Clec9A^{+}$ Flt3 MHC-II^{+} $XCR1^{+}$ Tissue specific: $CD8^{+}$ (lymphoid organs) $CD103^{+}$ (periphery)	BATF3 IRF8 DC-SCRIPT PU.1 ID2 NFIL3
cDC2s	Same as for cDC1s, but derived from pre-cDC2s	Survey local tissue and constitutively engulf extracellular material using multiple mechanisms of endocytosis (phagocytosis, macropinocytosis, and receptor-mediated endocytosis) MHC-II presentation Priming of naive CD4 T cells TNF, IL-6, and IL-23 secretion	$CD4^{+/-}$ $CD11b^{+}$ $CD11c^{+}$ $CD172a^{+}$ CD209 Flt3 MHC-II^{+}	IRF4 PU.1

Abbreviations: cDC1, type 1 conventional dendritic cell; cDC2, type 2 conventional dendritic cell; I-MAC, inflammatory macrophage; R-MAC, resident macrophage.

Remarkably, the cues that confer organ identity to developing embryonic macrophages exert a similar influence on monocytes, which are also plastic and follow similar tissue-specific differentiation programs as the fetal precursors upon taking residence in tissues (61). As a result, the monocyte-derived macrophages found at a particular location are largely equivalent to the macrophages generated before birth (10, 11, 24, 36, 37, 43, 44, 59, 62, 63). We henceforth refer to the macrophages generated before birth, and to those generated from bone marrow monocytes in the steady state, as resident macrophages (R-MACs) (**Table 1**). As we describe in Section 3, infection, inflammation, and/or stress at a particular site is accompanied with R-MAC losses and simultaneous recruitment of monocytes that also differentiate in situ into macrophages. We refer to the latter as inflammatory macrophages (I-MACs) (**Table 1**). While I-MACs acquire the same overall organ-specific identity as their R-MAC predecessors, their differentiation is now influenced by new factors released during inflammation. The I-MACs thus acquire genetic, phenotypic, and functional profiles (STAs) that set them apart from the R-MACs, and they stay in the tissues as a third group of self-renewing macrophages. The unifying principle of macrophage development, therefore, is their adaptability to conditions that vary in space (anatomy) and time (infection, inflammation, mechanical stress, etc.).

2.2.3. Functions of resident macrophages. R-MACs have two major functions (**Table 1**). The first is to maintain tissue homeostasis (10), which is accomplished by secretion of factors that promote local cell division and differentiation. R-MACs also eliminate by ingestion (efferocytosis) damaged cells or cells that have reached the end of their life cycle (64). Additional homeostatic functions recently described include cloaking tissue microlesions to hasten noninflammatory repair (65) and absorption of harmful microbial products (66, 67).

The second function of R-MACs, and the focus of this review, is immunosurveillance (68) (**Table 1**). The best-known activity of macrophages in host defense is phagocytosis and digestion of bacteria; other microorganisms; and particles bound by receptors that recognize microbial components (68) or host molecules that specifically bind microbes, such as opsonizing antibodies and complement (69–71). Obviously this activity takes place predominantly at exposed mucosal surfaces. Its importance is illustrated by the exacerbated bacterial infection that occurs in mice where viral infection impairs the phagocytic activity of R-MACs (72) or where a previous infection induces self-renewing R-MACs to reduce their phagocytic activity (73). Elimination of microbes and particles by R-MACs is in most cases immunologically silent in the sense that it is self-contained, not requiring the intervention of additional immune cell types or changes in tissue homeostasis associated with inflammation. This is important because inflammation is damaging and has the potential to cause, for instance, acute lung injury (74). It is beneficial for the host to eliminate as many bacteria as possible before triggering inflammation (15). In the lung, alveolar R-MACs patrol the lumen of alveoli (an extracorporeal space), capturing and thereby concealing bacteria to prevent detection by other cells that might induce adverse reactions (72). A similar role is played by R-MACs in other locations exposed to pathogens or environmental challenges, such as the gut, skin, and liver (36). In summary, R-MACs acquire during development an anti-inflammatory or at least hypoinflammatory STA and are dedicated to nonphlogistic concealment and elimination of bacteria.

When the level of infection surpasses the capacity for local containment, it is necessary to shift to a more aggressive response. We have described R-MACs as noninflammatory, but they can cooperate with other cells to cause inflammation by secreting type I interferons, IL-1β, and chemokines that recruit neutrophils and monocyte precursors of I-MACs (75–78). The specific mechanism that triggers the shift from silent to phlogistic pathogen elimination is not well defined; it may involve the amount of pathogen itself or the concomitant occurrence of pathogens and host

cell death (4, 15). This begs the question: Is the threshold of pathogen exposure at which R-MACs undergo this functional shift equally set at all sites of macrophage residence? It makes sense to assume that the R-MACs that patrol exposed surfaces should tolerate higher burdens of microbes before initiating inflammation than do those found in sterile internal organs. This provides a teleological basis for the inclusion of pro- and/or anti-inflammatory response modules as part of the STAs that developing R-MACs undertake to match the conditions of their tissue of residence. An example is the desensitization of macrophages of the colonic mucosa (79) or lung alveoli (51) to the bacterial component lipopolysaccharide (LPS). The mediators tailoring such STAs would be expected to be cells or molecules found only or predominantly within the tissue. There are multiple examples of such tissue-specific modulators. Alveolar basophils are locally imprinted by IL-33 and granulocyte-macrophage colony-stimulating factor (GM-CSF) (also known as colony-stimulating factor 2), and they in turn induce an anti-inflammatory STA in developing alveolar R-MACs (46). GM-CSF released by epithelial cells also induces the transcription factor PPAR-γ in alveolar R-MACs but not in other tissues (44). PPAR-γ adapts the metabolism of R-MACs to the lipid-enriched environment of alveoli and contributes to rendering the cells hypoinflammatory. Other molecules present in alveoli that contribute to maintaining local R-MACs in a resting state include CD200 (80); SIRP-α, an efferocytic receptor for CD47; and the surfactant proteins A and D (73, 81, 82). The microbiome is another major source of molecules that modulate macrophage STAs and their responsiveness to new challenges (14).

In summary, the full range of factors that regulate the inflammatory gene landscape of developing R-MACs to adapt to their tissue of residence remains incompletely characterized and is a major focus of research. Mechanistically, they affect not only the expression of transcription factors but also the epigenetic modifications that control gene accessibility, the synthesis of regulatory RNAs, and metabolic pathways involved in immune regulation (37, 83). Furthermore, as these factors change during and after infection, they provide the basis for the induction of training or tolerance/paralysis programs that alter the reactivity of macrophages over time (84), as discussed in Sections 3.1 and 4.

2.3. Development, Life Cycle, and Function of cDCs in Steady-State Mice

The cDC life cycle differs from that of macrophages in two fundamental aspects: cDCs are relatively short-lived [with a half-life of days (85, 86)], and they are continually replenished from bone marrow–derived precursors rather than by local self-renewal (12, 87–89) (**Figure 1**; **Table 1**). The rate of cDC generation is strongly and perhaps only dependent on the availability of the growth factor Flt3 ligand (Flt3L), recognized by the receptor Flt3 on bone marrow precursors (90). So-called pre-cDCs exit the bone marrow exhibiting few of the phenotypic or functional hallmarks that define cDCs, enter circulation, and access peripheral tissues and lymphoid organs. They undergo a few rounds of proliferation and produce offspring that acquire the phenotypic and functional features associated with the cDC lineage. In this regard, cDC differentiation follows a similar pattern to that described for macrophages. However, cDCs are not as plastic as macrophages, and all exhibit similar hallmarks regardless of the tissue where they undergo final development (91, 92), perhaps because their short life span limits the length of their exposure to niche cues. Nevertheless, as the functional properties of cDCs are acquired during final differentiation, there is a window of opportunity for modulation by local signals (93, 94), which can be mimicked in vitro by incubating bone marrow cDC cultures with growth factors (95). As the cDC network undergoes constant and fast renewal, it has the capacity to adapt quickly to changing spatiotemporal conditions. Although they are not an inextricable component of their tissue of residence in the way macrophages are, cDCs regulate secondary lymphoid organs and architecture through interactions with stromal cells (96, 97).

2.3.1. Hallmarks of the cDC lineage. Differentiated cDCs (**Table 1**): (*a*) express multiple receptors for pathogen- and/or tissue damage–associated molecular patterns; (*b*) are endowed with various endocytic mechanisms that allow them to capture pathogens, soluble antigens and cells; (*c*) express MHC-II molecules that present antigens synthesized by the cDCs themselves (endogenous) or antigens captured from the extracellular environment (exogenous) to CD4 T cells; (*d*) use their MHC-I molecules to present not only endogenous antigens, as most cells do, but also exogenous antigens (an activity known as cross-presentation) to CD8 T cells; (*e*) migrate from peripheral tissues or lymphoid organs to T cell areas within these organs; (*f*) express costimulatory and/or coinhibitory receptors that regulate naive T cell priming and the activity of primed T cells; and (*g*) secrete cytokines that also contribute to T cell regulation and play roles in inflammation and activation of innate lymphocytes (e.g., NK cells) (8, 12, 13). However, no cDC is endowed with all these features all the time, and it is important to introduce now two important concepts: first, the two major types of cDCs, namely cDC1s and cDC2s (98), and second, how the so-called activation process affects cDC properties (8, 99–101).

2.3.2. Two major types of cDCs: cDC1s and cDC2s. The bone marrow produces separate pre-cDC precursors for cDC1s and cDC2s, which disseminate via blood (98) (**Figure 1**). Both cDC types are found throughout the body, albeit in different proportions depending on location (12, 87–89). Each cDC type occupies a distinct niche within the peripheral tissue or lymphoid organ where it develops (102). cDC1s and cDC2s are often described as specialized at performing MHC-I cross-presentation to CD8 T cells and MHC-II presentation to CD4 T cells, respectively. This is a misleading concept that requires clarification (**Table 1**). In principle, the MHC-I and MHC-II antigen-presentation pathways of the two cDC subsets are identical (99, 103), as both produce and deposit on the cell surface equivalent amounts of MHC-peptide complexes (103, 104). What differs between the two cDC types is their ability to capture different types of antigen and their ability to deliver those antigens to the MHC-I and/or MHC-II presentation pathways (8). We return to these differences in the context of infection in Section 3.2.2. It suffices now to say that both cDC1s and cDC2s present antigens via MHC-II and that cDC1s possess two properties that cDC2s do not. First, they can efferocytose dying cells (105, 106). Second, cDC1s can deliver any type of endocytosed antigen to their MHC-I presentation pathway (i.e., cross-present) (107, 108). This capacity is conferred by a combination of properties that include, among others, unique antigen-trafficking pathways (109), delivery of antigens to endosomal compartments that are less acidic and proteolytic than those in cDC2s (110, 111), formation of hybrid compartments between endosomes and the endoplasmic reticulum (112, 113), and controlled disruption of endosomal membranes for release of the luminal content to the cytosol (114, 115), as reviewed in Reference 116. Combined with the differential distribution of cDC1s and cDC2s in secondary lymphoid organs (102), these differences dictate which of the two cDC types activates antigen-specific CD4 and/or CD8 T cells in the steady state and upon infection.

2.3.3. cDC migration and maturation in the steady state. Newly formed cDCs are traditionally termed immature (8, 100, 101, 117), but here we follow the recent recommendation to refer to them as resting (12), as this term is better aligned with the terminology applied to other cell types, including macrophages (**Figure 1**). Resting cDCs are characterized by high endocytic capacity, fast turnover of MHC-II molecules induced by ubiquitination (118, 119) and low expression of T cell stimulatory or inhibitory receptors and cytokines (101, 120, 121). Most of the resting cDCs that develop within lymphoid organs die quickly (their half-life is three to five days) and without undergoing further developmental changes (104) (**Figure 1**). The resting cDCs that develop in peripheral tissues live longer on average, but before dying they migrate via lymphatics to T cell

areas within the closest draining lymph node, guided by CCR7 recognition of chemokine gradients (122). Simultaneously the immigrating cDCs undergo activation [the process traditionally known as maturation (8, 100, 101, 117)], characterized by downregulation of endocytosis; higher expression of T cell costimulatory receptors (CD40, CD80, and CD86); and shutdown of MHC-II synthesis, ubiquitination, and turnover (118, 123). These changes result in a several-fold increase in surface expression of long-lived MHC-II molecules on activated cDCs. These cDCs can thus display to CD4 T cells peptides derived from antigens that were degraded in endosomal compartments before or during the activation process and that may no longer be available in the lymph node, presentation that probably lasts for as long as the cDCs themselves (123).

2.3.4. cDCs induce peripheral tolerance in the steady state. In uninfected mice, the spleen contains cDC1s and cDC2s that differentiated in situ and remain in a resting state. These are termed resident cDCs, although they do move within the spleen (102). Lymph nodes also contain equivalent resident cDC1s and cDC2s, in addition to their migratory counterparts, which have undergone activation (120, 121). All these cDCs present with their MHC molecules peptides derived from self-proteins, proteins produced by the commensal microbiome, or proteins that are ingested or inhaled and that are innocuous (8, 99) (**Figure 1**). Few T cells can recognize these antigens, and those that do die, become unresponsive, or (in the case of CD4 T cells) differentiate into regulatory T cells (Tregs), processes collectively known as induction of peripheral tolerance (12, 13, 124, 125). It is accepted that, in steady-state mice, activated (migratory) cDCs are involved in this process (120, 121), but it is not resolved yet whether resting (resident) cDCs also induce peripheral tolerance (101).

2.3.5. What regulates cDC activation in SPF mice? It is not clear why resident cDCs rest until they die, nor what causes activation of migratory cDCs in the steady state (101, 120, 121). Activation probably requires some stimulus that can be induced only in the periphery. It is not a microbial stimulus, because mice bred in germ-free conditions contain the same number of activated cDCs in their lymph nodes as do SPF mice (120, 126, 127). It is possible that migration and activation are imprinted in the cDC developmental program, but if that is the case, the activation component of that program is rarely turned on in resident cDCs. Activation may be triggered by disruption of contacts between migratory cDCs and the extracellular matrix or other cells (128, 129), but if that is the case, the question that remains is what induces cDCs to leave tissues even in germ-free mice (101). As we describe in Section 3, both resident and migratory cDCs can undergo immunogenic activation during infection, acquiring the capacity to prime naive T cells and initiate adaptive immunity (12).

3. MACROPHAGE AND cDC DEVELOPMENT AND FUNCTION DURING INFECTION

Macrophages and cDCs are equipped with a plethora of receptors for molecular structures found only on pathogens or released only during tissue damage (2–4). Their engagement triggers activation of the two cell types and signaling events that lead to genomic, proteomic, and ultimately functional changes that induce inflammation and adaptive immunity so as to eliminate the cause of activation and restore homeostasis. We are using here the same term—activated—to refer to both cDCs stimulated by infection and migratory cDCs that spontaneously migrate to local lymph nodes in the steady state, but in this case the activated cDCs acquire immunogenic features that their spontaneously activated counterparts lack.

All activated macrophages share some phenotypic and functional features, as do all activated cDCs, but they also vary depending on the type of stimulus (bacteria, viruses, vaccines, etc.), as each one engages a unique combination of receptors that initiate specific downstream signaling events. At the inflammation site, these primary inputs combine with secondary stimuli, comprising soluble cytokines and membrane receptors expressed by other cells that have themselves detected the original stimulus or other secondary stimuli. Any pathogen can express multiple primary stimulatory molecules, each able to induce secondary stimuli, so the complexity of signals at the infection site is formidable (4). It may even appear contradictory, as both activating (e.g., IL-12, interferons) and suppressive (e.g., IL-10, TGF-β) cytokines are produced simultaneously during inflammation even in response to a single Toll-like receptor (TLR) ligand (51). A third complication is that different types of macrophages and cDCs are programmed to respond differently even to the same stimulus. For instance, both cDC1s and cDC2s express TLR9, but only cDC1s secrete IL-12 upon stimulation with its ligand, CpG (130). Finally, the response of R-MACs and cDCs to any particular challenge varies depending on the conditions encountered by their precursors. Understanding how this complexity translates into specific immune mechanisms tailored to eliminate a defined pathogen at a particular site has been a major and far from complete objective of immunologists for decades. In this section we describe in general terms how macrophages and cDCs respond to infection as a transient alteration to the steady state. We also showcase how the differential behavior of the two cell types in response to the challenge can explain the fitness of the immune system to respond to a subsequent infection.

3.1. Generation of Monocyte-Derived Inflammatory Macrophages During Infection

Lung alveoli are an ideal site to study how R-MACs respond to infection or other challenges, and the effects of inflammation on macrophage development and differentiation. In SPF mice, alveoli are largely devoid of pathogens, partly because of inaccessibility. Indeed, the lower respiratory tract has traditionally been considered sterile, but it is now clear that it is under constant threat of infection (131). As described in Section 2.2.3, bacteria are ingested and degraded without triggering inflammation by alveolar R-MACs (36, 82, 132, 133). This steady state can be readily disrupted by intranasal or intratracheal inoculation of viruses, bacteria, fungi, allergens, or inflammatory compounds to track the innate and adaptive immune responses. Alveolar R-MACs initiate the response in cooperation with other cells by secreting inflammatory cytokines and chemokines that attract blood neutrophils and monocytes (36, 75–78, 82, 132, 133). The demand for blood monocytes is met in part by stimulation of emergency myelopoiesis in the bone marrow, a response of hematopoietic stem cells to pathogen or inflammatory mediators that causes the release of increased numbers of monocytes into circulation (134–136; reviewed in 137). Monocytes develop in situ into macrophages under the influence of inflammatory mediators (**Figure 2**). Because of their plasticity, instead of differentiating into R-MACs as they do in the steady state, they become I-MACs (**Table 1**), possessing a proinflammatory transcriptomic landscape (51). The program of monocyte differentiation into I-MACs can thus be considered a form of STA, in this case to a temporal change in the tissue environment rather than to an anatomical location. It is not clear whether the monocytes are prone to be inflammatory and the STA undertaken by I-MACs simply leaves open an inflammatory gene program that becomes closed during differentiation into R-MACs, or whether inflammation promotes in monocytes a shift to the more inflammatory profile displayed by I-MACs (83).

R-MACs and I-MACs coexist, at least at the beginning of infection, performing complementary activities (**Figure 2**). The R-MACs retain their anti-inflammatory and tissue-restorative profile (51) and function (36, 82, 132, 133). In their absence, exacerbated inflammation increases

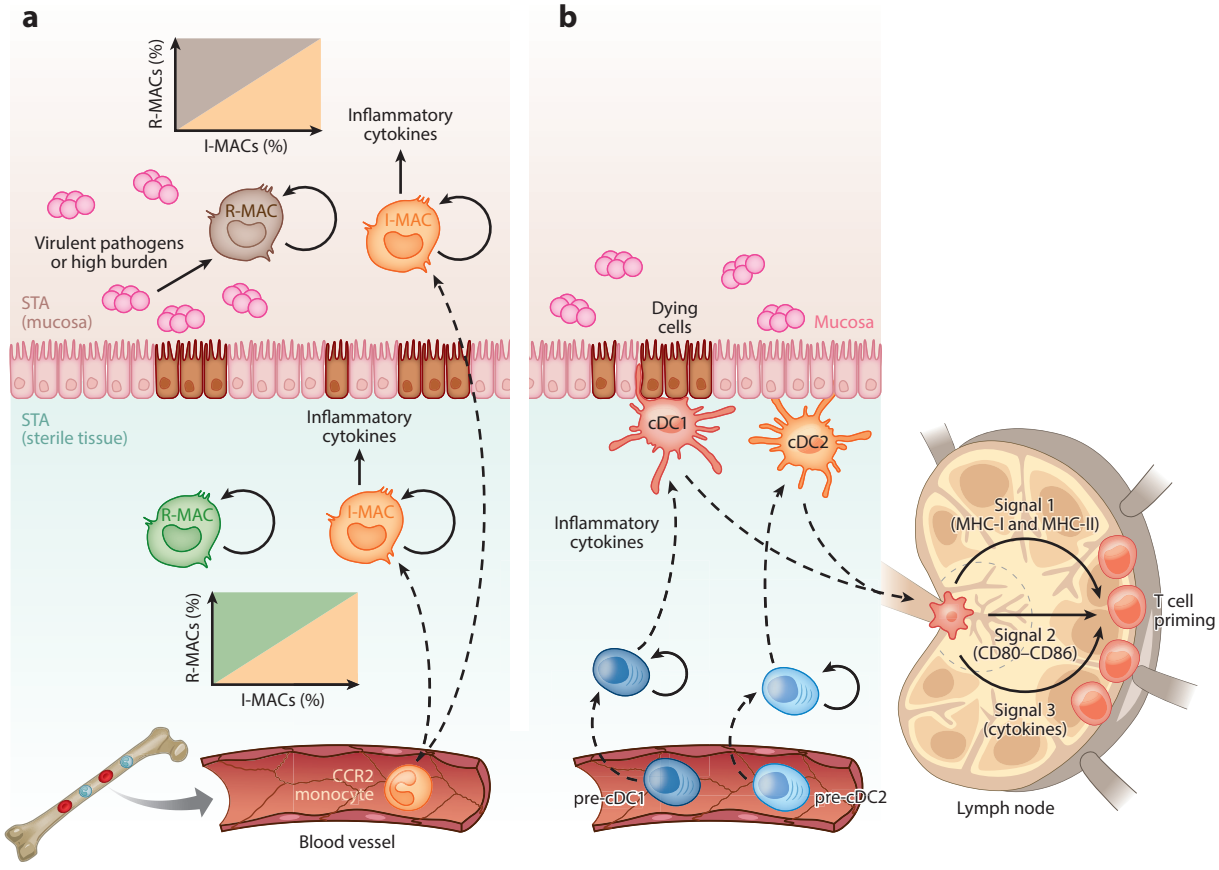

Figure 2

Macrophage and cDC response to infection. (*a*) When infection reaches a certain threshold, R-MACs contribute to initiation of inflammation that results in, among other events, recruitment of bone marrow–derived, circulatory CCR2$^+$ monocytes to the infection site. These monocytes develop into macrophages under the influence of inflammation, becoming I-MACs that acquire a proinflammatory STA, as opposed to the anti- or hypoinflammatory STA acquired by monocyte-derived R-MACs in the steady state (**Figure 1**). The I-MAC STA may be initiated in monocyte precursors in the bone marrow and finally established in the tissue. The R-MACs retain their hypoinflammatory STA, but they die in varying proportions. The niche left vacant by the R-MACs is occupied by the I-MACs, which become a self-renewing population. The more I-MACs are attracted and retained in the tissue (*green and orange* and *brown and orange graphs*), the more trained the local macrophages will appear after infection (**Figure 3**). (*b*) Encounter of pathogen-associated molecules causes cDC differentiation to an activated, immunogenic phenotype. Such cDCs provide type 1 (antigen presentation), type 2 (costimulation), and type 3 (cytokines) signals that induce T cell priming and initiation of adaptive immunity. The illustration shows activation of migratory cDCs at a mucosal surface, but a similar process is undertaken by resident cDCs that encounter pathogens in the spleen or lymph nodes. Renewal of the cDC network continues during infection. Abbreviations: cDC1, type 1 conventional dendritic cell; cDC2, type 2 conventional dendritic cell; I-MAC, inflammatory macrophage; R-MAC, resident macrophage; STA, spatiotemporal adaptation.

morbidity and mortality in virus- or bacteria-infected mice (138–140). The I-MACs drive inflammation and perform the classical activities associated with host defense, e.g., phagocytosis and killing of bacteria, secretion of microbicidal compounds, and elimination of apoptotic cells (68). Some of these activities require MHC-II antigen presentation. Macrophages are highly efficient antigen-presenting cells (141), so it is not surprising that I-MACs present antigens at the site of

infection (reviewed in 20). More controversial is the idea that they can migrate to lymph nodes and activate naive T cells, an archetypal cDC function (12, 13, 20). Although more research is needed to fully settle this matter, it is likely that in many, if not all, instances where I-MACs were described as migratory monocyte-derived cells performing T cell activation in lymph nodes, the cells carrying out this function were in fact cDCs that had acquired some I-MAC-like phenotypic features before emigrating from the infected site (20, 142).

Conceivably, the location and activities of R-MACs and I-MACs occur not in direct competition with each other but separately at subanatomical locations. Overall the balance at the site of infection is proinflammatory, and this condition increases with the number of monocytes that are recruited and become I-MACs. This shift is exacerbated by reductions in the number of R-MACs following rules that remain unclear. It does not appear to depend on the type of pathogen, because both maintenance (73, 143) and elimination (144–146) of R-MACs have been described during bacterial (73, 144) or viral (143, 145, 146) infection. It is not necessarily deleterious, as it may prevent dissemination of pathogens that can live inside macrophages (144, 147). It has also been proposed that R-MAC death by necrosis may promote beneficial inflammation (148). Notwithstanding the mechanism and its function, the outcome is that as the infection resolves, the ratio of R-MACs to I-MACs changes (**Figure 2**). The remaining R-MACs proliferate (73, 143), and their progeny retain the overall R-MAC profile, implying that the STA involved in the differentiation of monocytes into R-MACs is irreversible and heritable even in an inflamed environment. In fact, as the R-MACs replenish the tissue after infection, they can become less responsive to subsequent infections in response to local cues left over by primary infection (73) (**Figure 3**). The I-MACs also proliferate, occupying tissue niches left vacant by the R-MACs (146). The I-MACs initially maintain their proinflammatory profile but gradually shift to a less inflammatory STA. The mechanisms by which recruited monocytes rapidly become I-MACs and develop these functions upon arrival in the peripheral tissue remain largely unexplored. We revisit the consequences of R-MAC and I-MAC colonization and adaptation to the tissue environment remaining after the infection in Section 4.

The process we have described is based primarily on studies of lung infection, but it is largely applicable to macrophages in the gut, liver, and skin (36). The same can be said for macrophages in the spleen and lymph nodes (149, 150), which are rarely mentioned as sites of immunosurveillance but continually monitor the lymph and blood for pathogens (151), for instance, malaria parasites (136, 152). The R-MACs in each tissue have their own unique properties, established as STAs during development, and their location itself facilitates performance of certain specialized functions (149, 150). R-MACs in the peritoneum participate in the formation of epithelium-bound, fibrin-dependent immune cell aggregates that help to control bacterial infections (67). In addition to mediating inflammation, R-MACs in secondary lymphoid organs also capture and hand over to cDCs and B cells antigens that access spleen and lymph nodes through blood or, in the latter, lymph (153–156; reviewed in 157).

3.2. cDC Development and Function During Infection

When cDCs undergo activation in the context of infection, they contribute to the innate response by secreting cytokines, a function that can be nonredundant, as shown by the strong dependence on cDC1-produced IL-12 for *Toxoplasma gondii* elimination (158–160). IL-12 produced by cDC1s also induces IFN-γ secretion by NK cells, necessary for bacterial elimination (160, 161). However, the best-characterized function of cDCs is to initiate adaptive immunity by presenting antigen to and priming naive T cells (**Table 1**). The cDCs that are already present at the site of infection when the pathogen arrives are probably sufficient to initiate adaptive immunity, but

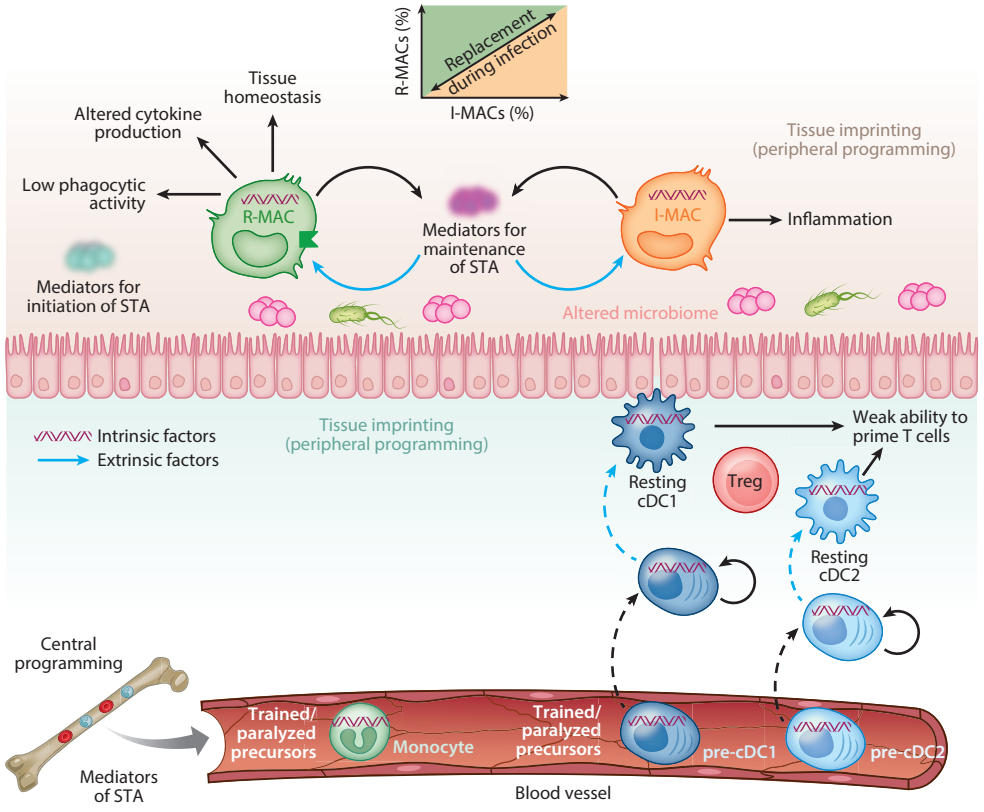

Figure 3

Generation and maintenance of trained, or paralyzed, macrophages and cDCs after infection. Following resolution of infection, hypoinflammatory R-MACs and proinflammatory I-MACs (*top*) coexist in different proportions according to the level of replacement that took place during infection (**Figure 2**). Both R-MACs and I-MACs self-renew, and their STAs are retained, or gradually altered, under the influence of local mediators. The cDC network maintains its normal rate of renewal (*bottom*). While the scenario appears equivalent to that before infection (**Figure 1**), R-MACs, I-MACs, and cDCs may have acquired STAs that render them more active (trained) or less active (paralyzed), following rules that remain poorly understood. Training and paralysis STAs are established by a combination of intrinsic (e.g., epigenetic) changes and extrinsic signals (e.g., cytokines produced by Tregs or by the macrophages and cDCs themselves). The STAs may be induced at multiple stages of cellular development, from the precursor stage in the bone marrow to the final differentiation stage in the tissues, though the local signals encountered during final differentiation probably play the dominant role. Abbreviations: cDC, conventional dendritic cell; I-MAC, inflammatory macrophage; R-MAC, resident macrophage; STA, spatiotemporal adaptation; Treg, regulatory T cell.

at least in some cases infection triggers emergency DC-poiesis (162), the enhanced production and release of pre-cDCs from the bone marrow, probably driven by increased Flt3L production (163, 164).

3.2.1. Developmental changes in activated cDCs that optimize antigen presentation and T cell priming. The reason cDCs excel at initiation of T cell immunity is because they are endowed with a unique combination of migratory; T cell–stimulatory; and antigen-capturing, -processing, and -presenting properties. We provide below a consensus view of these properties rather than a comprehensive review of an area where exceptions to general rules abound. It is important to

appreciate that many studies on the mechanisms of antigen processing and presentation in cDCs used as a model what we now know were not bona fide cDCs but monocyte-derived cells, most likely a type of macrophage, generated from bone marrow precursors cultured in the presence of GM-CSF (12, 13, 165). Some current assumptions based on studies of monocyte-derived cells may need validation using cDCs generated at least in Flt3L-supplemented bone marrow cultures (166) and ideally in vivo (167).

Activated cDCs have already endocytosed the infecting pathogens and/or infected cells, or do it soon thereafter owing to transient upregulation of endocytosis (168–170). They also undergo the two major processes described above for cDCs that become spontaneously activated in the steady state (**Figure 2**): They migrate to T cell areas of secondary lymphoid organs and increase expression of T cell costimulatory receptors (CD40, CD80, and CD86) and long-lived MHC-II molecules, some of which are loaded with peptides derived from the pathogen that caused cDC activation (8). Activated cDCs undergo an additional change: They secrete cytokines required for T cell priming and polarization (12, 13). Primed CD4 T cells become helper T cells of the most appropriate type (Th1, Th2, Th17) to fight the specific infection that caused cDC activation, or they become Tregs to restrain the response. Antigen presentation via MHC-I also enables activated cDCs to prime naive CD8 T cells and, together with helper T cells, induce their differentiation into cytotoxic T lymphocytes. A notorious property of activated cDCs is that by the end of their differentiation process they have downregulated antigen uptake (171–173), delivery of antigen to the MHC-I cross-presentation pathway (173, 174), and MHC-II synthesis (99, 175, 176). These effects tune the activated cDCs toward efficient presentation of MHC-peptide complexes generated during activation but make them refractory to present antigens encountered subsequently (173, 175). Downregulation of antigen capture and presentation is not deleterious when a pathogen causes activation of a limited number of cDCs, but severe infection can lead to excessive, simultaneous activation of cDCs and inability to mount immune responses against other pathogens, contributing to sepsis-induced immunosuppression (173, 175, 177, 178). It is pertinent to note that changes in synthesis, endocytosis, and MHC-II trafficking that cDCs undergo during activation are not unique to this cell type, as they are also observed in embryonic skin macrophages (Langerhans cells) (179), monocyte-derived macrophages (180, 181), and, partially, B cells (182) and plasmacytoid DCs (176).

The understanding of the process of cDC activation we have described assumes the only source of stimulation is direct contact with a pathogen component, but as mentioned earlier, this occurs in a context of inflammation where other inputs influence the properties of the activated cDCs in ways that remain incompletely understood (183). At one extreme of the spectrum, it is likely that many cDCs respond to inflammatory cytokines without having encountered the pathogen at all. These indirectly activated cDCs do not secrete signal-3 cytokines (184, 185) but retain their ability to capture and present antigens, unlike their directly activated counterparts (181, 185). However, the previous encounter of inflammatory signals conditions this secondary wave of activated cDCs to produce an altered set of cytokines, which may induce a different polarization program on primed T cells (185). A study has shown that type I interferons imprint cDC2s with cDC1-like functions (142). Acquisition of cDC1-like cross-presentation capacity by cDC2s stimulated via FcR with immunocomplexes has also been described (186). These observations might be interpreted as the induction of STAs in responding cDCs, resembling the differential imprinting that monocytes undergo as they develop into different forms of R-MACs in different locations (space) or into I-MACs during inflammation (time). By inference, the cDCs that drive T cell priming during infection may comprise more varied and complex profiles, imprinted by different combinations of pathogen and inflammatory signals than those observed in studies that use a single stimulus to favor a specific but artificially biased differentiation program (187). Furthermore, it

cannot be excluded that induction of cDC1-like properties in cDC2s occurs at a pre-cDC stage of development rather than on fully developed cDC2s, just as cDC1s gain their characteristic capacity to cross-present during transition from pre-cDC1s to cDC1s under the influence of external stimuli (95). Single-cell analysis of cDCs of animals exposed to different levels and types of pathogen infection may reveal more complex patterns of cDC differentiation and function than are currently recognized (188).

3.2.2. Antigen presentation and T cell–stimulatory specializations of cDC1s and cDC2s. When considering the cDC response to infection it is also important to address cDC diversity (see Section 2.3.2; **Table 1**). Establishing whether the different cDC types contained in an SPF mouse are poised to prime CD4 T cells, CD8 T cells, or both in response to different challenges has been the subject of intense investigation. Up to four cDC populations might perform this function: lymphoid organ–resident cDC1s and cDC2s in both spleen and lymph nodes and their migratory counterparts in lymph nodes only. As T cell priming requires the antigen-presenting cell to establish an immunological synapse with an antigen-specific T cell, studies on this matter have addressed one or more of the following questions. (*a*) Which cDC produces or captures the pathogen antigen? (*b*) How efficiently do those cDCs present the antigen via MHC-I and/or MHC-II molecules? (*c*) Which of the cDCs that present the antigen interacts with, and primes, antigen-specific CD4 and/or CD8 T cells? The answers are best summarized in the context of the type of antigen under consideration (8):

- Antigens from viruses that infect cDCs. By definition, such viral antigens are endogenous and are efficiently presented via MHC-I by the infected cDCs. Endogenous viral membrane proteins are degraded in endosomal compartments and also presented via MHC-II, as are cytosolic antigens delivered to such compartments by autophagy (189). Hence, any infected cDC1s or cDC2s, resident or migratory, might prime CD4 and CD8 T cells against the virus (8). Owing to their specific localization in different regions of the lymphoid organs, cDC1s are better poised to induce cytotoxic CD8 T lymphocyte responses and the cDC2s to induce CD4 Th cells. Naturally, this description assumes that the infecting virus does not express immunoevasins that disable antigen presentation (190).
- Viral antigens that are synthesized by non-cDCs and that are not secreted. Presentation of such antigens requires efferocytosis of the antigen-expressing cell (64). This is carried out by cDC1s (105, 106), which can then (cross-)present the antigen via MHC-I and MHC-II (191). If the cellular source of antigen is confined to a peripheral tissue, only the migratory cDC1s can capture and present it. Resident cDC1s can obtain the antigen from the migratory cDC1s and also present it (192). If the cellular source of antigen occurs primarily in blood or can reach lymph nodes via lymph, presentation will be carried out mainly by resident cDC1s (107, 108, 193). In this scenario cDC1s are the primary inducers of CD4 and CD8 T cell responses.
- Other exogenous antigens. These include soluble proteins, bacteria, vaccines, and particles endocytosed alone or together with immunoglobulin or complement following opsonization. Capture of some of these antigens may require specific receptors expressed only or preferentially by either cDC1s or cDC2s, whereas other antigens may be captured by both cDC types equally. In most cases cross-presentation and CD8 T cell priming are carried out only by cDC1s (8, 107, 108, 194). Both cDC1s and cDC2s can perform MHC-II presentation and prime CD4 T cells, although given a similar efficiency of antigen capture, cDC2s are generally more efficient than cDC1s (8, 107, 108, 194).

The antigen-presentation and T cell–priming abilities of cDC1s and cDC2s are well aligned with their pattern of expression of receptors for pathogen-associated molecules, with the type of

polarization they induce in T cells, and with their location in lymphoid organs to initiate immunity against different pathogen classes. Specifically, cDC1s are particularly adept at initiating immunity against viruses and intracellular bacteria (cytotoxic T lymphocyte and Th1 responses), and cDC2s against multicellular parasites and allergens (Th2 responses) and extracellular bacteria (Th17 responses) (12, 13). However, as mentioned above, it cannot be excluded that these specializations can be reassigned by combinations of different direct and/or indirect stimulatory signals at sites of infection (142).

3.2.3. Cooperation between cDCs and B cells. Although not as extensively studied as the induction of T cell responses, cDCs participate in B cell antigen acquisition and, possibly, activation (195, 196; reviewed in 197). A recent report has described trogocytic transfer of antigen-presenting molecules and other receptors from cDCs to B cells, in particular marginal zone B cells (198).

4. MACROPHAGE AND cDC DEVELOPMENT AND FUNCTION AFTER INFECTION

The classical view of the innate immune response following resolution of infection is that it rapidly restores homeostasis, the conditions that existed before infection. It is now clear that this is a simplistic view (199). Depending on the experimental model used, pathogen exposure can improve the response to a subsequent pathogen—a phenomenon known as immunological training (200)—or increase susceptibility to subsequent infections, termed tolerance or paralysis (201). Both phenomena have wide-ranging implications that help to explain, respectively, heterologous protection (202) and induction of immunosuppression following sepsis, trauma, or other triggers of the so-called systemic inflammatory response syndrome (SIRS) (201, 203, 204).

Training and paralysis can affect multiple cell types (200), but here we focus on cDCs and macrophages (**Figure 3**). For instance, the response of alveolar macrophages to viral or bacterial pneumonia in mice that have recovered from a prior lung infection is different from that in mice that have not been previously infected (73, 93, 146). Paralysis has also been described in cDCs as causing impairment of the adaptive, along with the innate, immune response (93). The term tolerance is normally used in this context to refer to an inability to respond to LPS or other TLR ligands (200, 205), but functional defects observed in paralyzed macrophages and cDCs extend to impaired phagocytosis, antigen presentation, and cytokine secretion (73, 93). We propose that training and tolerance or paralysis are STAs analogous to those that both cell types undergo in the steady state or during immune responses. Indeed, what we call paralyzed cells may not be impaired to carry out any function but adapted to respond differently when compared to their steady-state counterparts (e.g., from type 1 to type 2 immunity) or to carry out nonimmune functions such as restoration of tissue homeostasis (145). Nevertheless, we continue to refer to this STA as paralysis for convenience, as it has been mostly studied in the context of immunosuppression after sepsis or trauma (201).

This section is not divided into subsections for macrophages and cDCs, because we think it is more informative to describe how a particular mechanism may affect either cell type. The main questions we will consider are the following: (*a*) Does the type of infection determine whether the postinfection STAs will lead to training or paralysis? (*b*) In the case of macrophages, do R-MACs, I-MACs, or both acquire trained/paralyzed STAs, and do they remain in tissues after infection? (*c*) Are the STAs initiated in bone marrow monocytes, pre-cDCs, or even earlier precursors, or are they induced in local tissues? (*d*) What is the relative contribution of intrinsic imprinting versus extrinsic factors to maintenance and duration of STAs after infection?

4.1. Different Infectious Agents Can Induce Training or Paralysis

It is not clear why the outcome of infection is in some cases training and in others paralysis. The field is dominated by in vitro experimental systems where monocytes are subjected to various stimuli that can help define mechanisms leading to training or tolerance, but these approaches do not predict in vivo outcomes (199, 200). Macrophage training can be induced in animals by systemic administration of the fungal component β-glucan (206) and the bacillus Calmette-Guérin vaccine (207). In contrast, LPS or CpG administration induces tolerance (73, 93). In these cases, training or tolerance can be seen as a response to a single exposure to a microbial compound rather than to a complex pathogen. Regarding infectious agents, viral, bacterial, or fungal pneumonia has been shown to induce macrophage training in the lung (143, 146, 206). Pulmonary fungal infection also caused training of cDCs (208). However, other studies have shown that viral or bacterial pneumonia induces paralysis of lung macrophages and cDCs (73, 93, 209), systemic administration of *Mycobacterium tuberculosis* impairs training (210), and malaria infection causes macrophage and cDC paralysis in the spleen (136, 211). The field is undergoing rapid development, and it is too early to unify all the observations under a coherent model.

4.2. Resident Versus Inflammatory Macrophages After Resolution of Infection

A possible explanation for divergent macrophage STAs after infection is the magnitude of replacement of R-MACs with I-MACs during infection and the persistence of the latter. Infection can trigger both R-MAC losses and monocyte recruitment and differentiation into I-MACs (**Figure 2**), which stay in the tissue as a self-renewing population (146, 212) (**Figure 3**). Since the STA of I-MACs is more inflammatory than the STA of R-MACs, the more replacement there is, the more trained the combined population will appear (83, 133). This scenario is supported by a study in which viral pneumonia caused almost complete replacement of alveolar R-MACs with I-MACs, which remained after infection as self-renewing macrophages (146). In contrast, two other studies reported that viral or bacterial pneumonia did not cause replacement of alveolar R-MACs with I-MACs (73, 143). In one of these studies the R-MACs became trained (143), a surprising result because the STA of R-MACs is considered largely irreversible (37, 83). In the second study, the R-MACs acquired a paralyzed STA, contributing to immunosuppression and increased susceptibility to secondary pneumonia after infection (73). Interestingly, in one study the extent of R-MAC replacement in response to malaria infection varied among tissues, indicating that a single infection might lead to various levels of training STAs at different anatomical locations (212). Population replacement cannot explain changes in cDCs, because these cells do not self-renew in tissues and maintain their fast turnover during and after infection (85, 93).

4.3. Are the Postinfection STAs Imprinted in the Bone Marrow or in the Periphery?

The site and developmental stage at which infection triggers STAs in cDCs and macrophages are also controversial. Several studies have reported that infection or pathogen products can induce epigenetic programming in hematopoietic stem cell bone marrow precursors that later convert into trained I-MACs and cDCs (134, 210, 213, 214) (**Figure 3**). This mechanism would cause seeding of preprogrammed cDC precursors throughout the body, potentially causing development of trained or paralyzed cDCs in all tissues. However, pneumonia caused cDC paralysis in the lung but not in the spleen (93), arguing against a systemic process. Induction of paralyzed cDCs in a model of malaria infection also occurred in peripheral tissues, not the bone marrow (136). The case of macrophages is different. Monocytes can infiltrate and generate I-MACs only

at inflammation sites, so it would be possible for precursors that underwent STAs in the bone marrow to become trained, self-sustaining I-MACs only in the infected location. The alternative site for STA induction is the tissue itself. Local signals present during infection (**Figure 2**) or left over after its resolution (**Figure 3**) might contribute to induction of a training or paralysis STA in I-MACs, potentially overriding any preprogramming undergone by their monocyte precursors in the bone marrow. By definition, STA induction in R-MACs can happen only locally, and this was the conclusion of two studies that reported training (143) and paralysis (73) of alveolar R-MACs after infection. Based on its restriction to the location where infection took place, this is also the most likely mechanism responsible for generation of paralyzed cDCs (93) (**Figure 3**).

4.4. Intrinsic Versus Extrinsic Mechanisms in Maintaining Spatiotemporal Adaptations After Infection

It is not clear whether the duration of training or paralysis STAs depends on external signals. Trained I-MACs induced by influenza virus infection gradually adopted an R-MAC-like STA over time, possibly without the intervention of external mediators (83, 133, 146). However, studies of R-MAC and cDC paralysis in the lung suggest that extrinsic tissue signals play an important role in STA maintenance (73, 93). When alveolar R-MACs from an infected mouse were transferred to a noninfected recipient, their progeny adopted the same functional properties of the recipient R-MACs. In contrast, when alveolar R-MACs from a noninfected animal were transferred to an infected recipient, both the transferred and the host R-MACs were paralyzed long after clearing the infection (73). Tissue signals thus appeared not just to establish (**Figure 2**) but also to maintain (**Figure 3**) the STA that caused R-MAC paralysis. This aligns well with the observation that R-MAC identity and hypoinflammatory profile are established and maintained by interactions with the local environment (see Section 2.2.3) (11, 36, 37). In the case of cDCs, their short half-life and continuous replenishment with bone marrow precursors imply that long-term changes circumscribed to a particular location must be caused by extrinsic signals acting locally on the developing cDCs. In the lung, TGF-β and Tregs have been shown to play a role in cDC paralysis (93). Engagement of SIRP-α expressed by R-MACs early in infection can trigger the establishment of paralysis signals (**Figure 2**), even if the maintenance of the paralysis program after infection is no longer dependent on this receptor (73) (**Figure 3**). The implication of this mechanism is that infections leave a legacy of local signals that induce or maintain STAs. Interestingly, this model implies that it might be possible to induce subanatomical adaptations so that, for example, some lung regions might contain macrophages and cDCs with a trained bias while others might contain paralyzed cells. This is easier to envisage for macrophages because they stay on-site, but subanatomical programming is also feasible in cDCs because it has been shown that the progeny of pre-cDCs remain clustered, implying that each group of daughter cells can be exposed to different tissue microenvironments and develop under the influence of the signals contained in that location (162). Subanatomical programming would result in the generation of a mosaic of macrophage and cDC specializations within the organ.

5. OF WILD MICE AND HUMANS

Virtually all the studies we have reviewed are based on SPF mice, and it is pertinent to ask, if an SPF mouse were exposed from birth to multiple infections and environmental insults, would its cDC and macrophage network continually change through life via STAs? The most reasonable answer is yes, implying that in such a mouse the functional properties of its cDCs and macrophages at different anatomical locations might be a combination of phenotypes more or less biased toward training or paralysis. Furthermore, these biases would change throughout the life of the

animal. There is good evidence to indicate this is the case, as we summarized in Section 2.1. Studies comparing response to infection in SPF and wild mice are scarce, but they do show different outcomes (29, 30, 32, 35). It is difficult to know to what extent this can be attributed to differences in macrophages and/or cDCs, as variations in other components of the immune system, the microbiota, or even non-immune-related systems may play a role too. This is an important question for future studies.

Characterization of human macrophages and cDCs shows that most of the conclusions based on studies of the mouse system are applicable to humans, including those about macrophage origin (9), persistence of embryonic R-MACs (37), cDC types and function (215), and plasticity of monocytes, which can differentiate into trained or paralyzed macrophages (200, 216). Analysis of circulating monocytes and cDCs in trauma patients reveals paralysis marks that resemble those found in mice (73, 93). Obviously differences between the murine and human systems exist, but to what extent are these due to interspecies variation as opposed to differences in exposure to the environment? When the microbiota of SPF mice is normalized to resemble that of wild animals, not only do mice have an immune system that is more human-like (32) but their immune response also recapitulates human traits (30, 31). It follows that STAs must continually tune the macrophage and cDC network of humans to different anatomical locations and changes in the environment, and these STAs may explain differences between species and between individuals. We already know that certain stimuli can endow mouse cDC2s with cDC1-like properties, notably cross-presentation (142, 186); perhaps this is the reason both cDC1s and cDC2s can cross-present in humans (217). This is speculative at present, but it is inescapable that if we want to fully understand the function of macrophages and cDCs in immunosurveillance, we need to understand how their tissue environment and past pathogen encounters affect their functions. Such a notion extends and refines the concept first formulated as the hygiene hypothesis (218, 219). Further progress will require deeper characterization of the mechanisms that impart STAs and the correlations that exist between the type and magnitude of a particular infection and the effects on subsequent responses to new challenges.

6. CONCLUDING REMARKS

"Paradigm shift" is a somewhat abused phrase, but there is no alternative to describe the impact the last decade of research on the role of the local environment has had on our understanding of immunity. The descriptions of innate lymphoid cells (220) and tissue-resident memory T cells (221) are just two prominent examples. The advances in our understanding of macrophage and cDC origins, development, and roles in setting up the conditions for the local immune response fall in the same category. They have provided a foundation to better understand tissue homeostasis and immunosurveillance and opened avenues to harness their benefits while avoiding their deleterious outcomes. At the time of writing, coronavirus disease 2019 (COVID-19) represents a dramatic example of an infectious disease where this new knowledge might make a lasting impact (see the sidebar titled Roles of Macrophages and Dendritic Cells in Severe COVID-19). The capacity of macrophages and cDCs throughout the respiratory tract to detect SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) may determine the swiftness and potency of the response. Could the STAs of the cells of each individual dictate the initial susceptibility to the disease? The most deleterious aspect of said response, a dysregulated cytokine storm in a small cohort of patients, may originate from the predisposition of the macrophages and cDCs of those individuals to respond excessively—runoff training. The fact that the overwhelming majority of these patients are among the aged raises the question as to whether past encounters with certain pathogens cumulatively contribute to priming their macrophage-cDC network toward a pathological response. Perhaps

ROLES OF MACROPHAGES AND DENDRITIC CELLS IN SEVERE COVID-19

Since 2020, more than 200 million people worldwide (probably a gross underestimate) have been infected with SARS-CoV-2, the causal agent of COVID-19, resulting in more than 10 million deaths as of December 2021. While most cases of infection result in mild disease and full recovery, elderly people and those with comorbidities are at high risk of severe pneumonia, acute respiratory distress syndrome (ARDS), long-term sequalae and death (222–224). Severe disease is not as much a consequence of viral infection per se as it is of the SIRS that develops in these patients. Vaccines have proven phenomenally successful at containing viral transmission and, especially, preventing acute infection and hence severe COVID-19 (225), but the regular emergence of immune escape variants is a constant threat. Antiviral drugs have shown limited efficacy against development of SIRS and, again, may become ineffective against new variants of the virus. In this context, therapies directed toward the host immune response to prevent, mitigate, or shorten SIRS in COVID-19 patients are a high priority. Indeed, several immunotherapies that reduce the lung inflammatory response by interfering with cytokine signaling have demonstrated efficacy to treat severe COVID-19 (226–228). Can we harness the knowledge recently acquired on macrophage and cDC development and function to prevent SIRS altogether?

Macrophages and cDCs appear to be major drivers of SIRS in COVID-19 (229). Several immunological features characteristic of other severe respiratory conditions, such as increased expression of CCR2 on cDC2s (230) and airway infiltration with $CCR2^{+}$ monocytes recruited by local CCL2 production (216), are also observed in COVID-19 patients. The evolution of COVID-19 pneumonia toward ARDS is specifically associated with an interferon-stimulated gene signature, downregulation of MHC-II expression, and CD163 upregulation in circulating monocytes (216, 231). Patients with severe COVID-19 pneumonia showed accumulation of I-MACs in respiratory fluids compared to healthy controls (232). While these findings indicate a role for macrophages and cDCs during the early inflammatory phase of COVID-19, studies addressing long-term alterations in both cell types in survivors are still lacking. Investigating macrophage and cDC reprogramming following recovery from COVID-19 is critical because a significant portion of patients develop lung fibrosis, suffer prolonged respiratory symptoms, and remain susceptible to secondary infections for months after hospital discharge (222).

In addition to helping manage COVID-19 patients, detailed characterization of R-MACs, I-MACs, and cDCs during and after recovery from severe infection may also provide important clues applicable to other conditions where SIRS plays a pathological role. These include respiratory infections, other forms of sepsis, and even sterile inflammatory conditions such as severe trauma (178, 201, 233, 234).

those individuals have an unusually high ratio of I-MACs to R-MACs? Therapies to ameliorate severe COVID-19 are few at the time of writing, and of limited efficacy. The phenomenal number of ongoing studies both in the clinic and in the basic research laboratory may generate new approaches. Profiling of circulating monocytes might identify patients susceptible to hyperinflammatory responses. Therapeutic strategies to "untrain" macrophages and cDCs, to force quick replacement of trained I-MACs with less-inflammatory ones, or to eliminate the tissue signals that maintain training might be effective. New RNA-based vaccine technology has transformed the management of the COVID-19 pandemic and promises to revolutionize infectious disease prevention in general. The role of macrophages and cDCs in the induction of protective immunity by this new class of vaccines remains largely unknown. Filling this gap will undoubtedly be another driver of research on the two cell types. These questions are applicable to immunity against other infectious diseases and cancer, and the answers may also provide solutions to treat autoimmunity, allergy, and chronic inflammation. We hope with this review we inspire other researchers to test new ideas and to consider the potential of combining studies on macrophages and cDCs in their experimental systems, as we are certain such approaches will yield lasting rewards.

DISCLOSURE STATEMENT

A.R., J.D.M., and J.A.V. are inventors on a patent on the use of IL-12 to treat infection (WO2019016109A1), and A.R. is inventor on a patent on modulation of macrophages during inflammation (WO2020260281A1).

ACKNOWLEDGMENTS

A.R. is supported by the European Union's Horizon 2020 research and innovation program under grant agreement number 847782 and the Agence National de la Recherche (ANR) PROGRAM project. J.A.V. is supported by a Principal Research Fellowship (1154502), a program grant (1113293), and a project grant (1163090) of the National Health and Medical Research Council of Australia. J.D.M. is supported by an Australian Research Council Future Fellowship (FT180100521), an Australian Research Council Discovery Project (DP190101242), and a National Health and Medical Research Council of Australia Ideas Grant (2000683).

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