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Monocyte activation in systemic Covid-19 infection: Assay and rationale

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ABSTRACT

Mononuclear phagocytes are a widely distributed family of cells contributing to innate and adaptive immunity. Circulating monocytes and tissue macrophages participate in all stages of SARS COVID-19. They contribute to comorbidities predisposing to clinical infection, virus resistance and dissemination, and to host factors that determine disease severity, recovery and sequelae. Assays are available to detect viral infection and antibody responses, but no adequate tests have been developed to measure the activation level of monocytes and tissue macrophages, and the risk of progression to a fatal hyperinflammatory syndrome. Blood monocytes provide a window on the systemic immune response, from production to tissue recruitment, reflecting the impact of infection on the host. Ready availability of blood makes it possible to monitor severity and the risk of potentially lethal complications, by developing tests to assess the status of monocyte activation and its potential for further inflammatory dysregulation after recruitment to tissues and during recovery.
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1. INTRODUCTION

COVID-19 infection presents a risk of severe clinical outcome due to a dysregulated inflammatory syndrome [1,2] generated by Mononuclear Phagocytes [3], a major source of pro-inflammatory secretion products. Evidence from influenza [4,5] and previous SARS and MERS [6] patients, indicates that blood monocytes respond to tissue encounters in a two-stage mechanism of activation [7], which is not specific to the initiating infection. This is particularly relevant in COVID-19 where age and predisposing comorbidities enhance the risk of a severe outcome [8,9]. Tests are available to detect infectious virus and anti-viral antibody, but RNA alone is not proof of active infection and antibodies may not neutralize, but enhance infection [10]. In order to stratify patients at risk of a hyperinflammatory storm and prolonged recovery, we developed a novel blood test to assess their level of monocyte activation. It uses the canonical CSF1R–CSF1 growth factor receptor [11], to characterize all monocytes in blood, Fig. 1. Further steps include measures of priming during haematopoiesis along myeloid differentiation pathways mediated by CSF2R [12] and CSF3R, and testing their full activation potential by selected stimuli, in vitro, as surrogates of activation in tissues. Tests can also be used to evaluate macrophage contributions to efficacy of vaccines, anti-viral and anti- inflammatory agent trials, to diminish the risk of treatments and facilitate repair.

Although the airways represent a focus of COVID-19 infection and immediate threat to the host, the virus can disseminate systemically and have a profound impact on the body. While epithelial and endothelial cells are the major targets of viral infection through expression of the ACE2 receptor [13,14] innate and adaptive immune responses of the host contribute critical resistance and pathogenic responses at all stages of disease. Effector cells of the Mononuclear Phagocyte System (MPS), whether they become infected [15] or not, depend mostly on recruitment of bone marrow-derived monocytes to tissues [16-18] rather than resident macrophages of embryonic origin [16,19].

Abbreviations: ace, Angiotensin converting enzyme; ade, Antibody dependent enhancement of infection; adi, Antibody dependent enhancement of inflammation; ards, Acute respiratory distress syndrome; axl, Receptor tyrosine kinase; bcg, Bacille calmette guerin; bmi, Body mass index; covid-19, Coronavirus-2019; crp, C-reactive protein; csf1r, Colony stimulating factor 1(macrophage) receptor; csf2r (gm-csf), Colony stimulating factor 2 (granulocyte macrophage) receptor; csf3r (g-csf), Colony stimulating factor 3 (granulocyte) receptor; dc, Dendritic cell; igf, Insulin-like growth factor; ifn, Interferon; iris, Immune reconstitution inflammation syndrome; itam, Immunoreceptor tyrosine-based activation motif; itim, immunoreceptor tyrosine-based inhibition motif; IVIg, intravenous immunoglobulin; MAS, Macrophage activation syndrome; MERS, Middle East respiratory syndrome corona virus; MERTK, MER proto-oncogene tyrosine kinase; MCP-1, Macrophage chemotactic protein 1; NETosis Neutrophil extracellular trap; MPS, Mononuclear Phagocyte System; NK, Natural killer cell; SARS, Severe acute respiratory syndrome; PMN, Polymorphonuclear leukocyte; STING, Stimulator of Interferon genes; TAM, Tumour associated macrophage; TGF, Transforming growth factor; TLR, Toll-like receptor; TMPRSS2, Transmembrane protease, serine 2; TNF, Tumor necrosis factor

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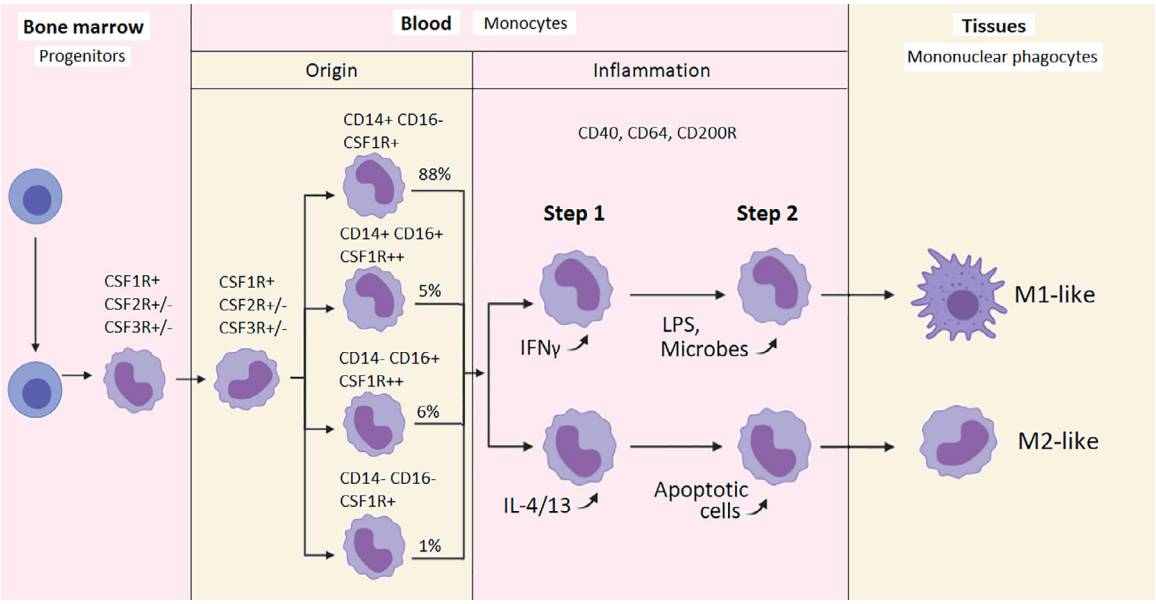

Fig. 1. Monocytes represent a window between bone marrow and tissues. Blood monocytes are circulating intermediates between haematopoiesis in bone marrow and recruitment to tissues in response to turnover of resident macrophages of embryonic origin, and to increased demands following tissue inflammation, infection and malignancy. The CSF1 Receptor is a pan-monocyte marker expressed by all subsets of monocytes defined by CD14, CD16 and DCs, lacking both of these antigen markers. Together with CSF2R and CSF3R, these three lineage differentiation markers enable identification of distinct streams of monocytic, granulocytic and Dendritic cell types of monocyte differentiation. In response to the prototypic TH1 or TH2 cytokines, IFNγ and Interleukin 4/13, monocytes are polarized to distinct M1-like, classically activated, and M2-like, alternatively activated macrophages. Each becomes fully activated by further local phagocytic stimulation by microbes and apoptotic cells, respectively. CD40 and CD64 are M1-like markers, where CD40 is for detecting TLR4 pathway and CD64 is for detecting IFNγ activation, while CD200R is an M2-like marker. Other markers can be introduced as required.

Mononuclear phagocytes collaborate with other immune and non-immune pathways to mediate local and systemic anti-viral resistance and recovery, while also promoting morbidity and mortality. The MPS is a major contributor to the hyperinflammatory and procoagulant secretion syndrome, as demonstrated in earlier SARS syndromes as well as COVID-19 infection [20]. Ready access to peripheral blood samples from those at risk, provides a window on the dynamics of infection and their monocyte activation status, a guide to clinical diagnosis, treatment and prevention.

2. The mononuclear phagocyte system (MPS)

The MPS is a dispersed organ, present throughout the body, with distinctive properties in every organ [21]. Tissue resident macrophage populations in the adult derive from embryonic progenitors and differ markedly in phenotype from bone marrow-derived monocytes, which are recruited to tissues in response to increased demand, for instance infection and tissue damage [19]. Monocyte- derived macrophages are the main generators of inflammation in COVID-19 [2,17,18,20,22-24]. These mononuclear phagocytes express a broad repertoire of plasma membrane and intracellular receptors, serving as sensors of micro-organisms, dying cells and soluble products, to mediate recognition, signaling, migration and activation [19]. Monocytes and macrophages are professional phagocytes, utilizing Fc, complement, Toll-like and non-opsonic lectin-like and scavenger receptors for ingestion, but are also highly active biosynthetic and secretory cells, contributing to inflammation, innate and adaptive cellular and humoral immunity and antimicrobial defences [21]. While promoting tissue homeostasis and repair, they also induce tissue injury, the proverbial two-edged sword. They interact readily with other cells through contact and soluble mediators including cytokines, chemokines and enzymes that activate plasma coagulation and complement cascades [24]; in addition, they generate reactive oxygen, nitrogen and arachidonate metabolites implicated in host inflammation and resolution [25]. By analogy with lymphocytes, their activation status is characterized for convenience as M1-type (pro-inflammatory) and M2-type (anti-inflammatory, reparative) [7]. Unlike T and B lymphocytes, mononuclear phagocytes are antigen non-specific, and only display a transient form of memory [26], primed to adapt to secondary challenges such as phagocytosis and microbial stimuli by further activation of potent secretory and cytotoxic pathways [7]. The level of activation covers a spectrum of effector mechanisms, constrained by inhibitory cytokines such as IL-10, IL-1 receptor antagonist [27] and transforming growth factor beta TGF-β, and by anti-inflammatory glucocorticoids and prostaglandins.

3. COVID-19 infection

3.1 It is customary to divide the course of the infection into different stages: Preinfection (co-morbidities), asymptomatic, early clinical, severe clinical (acute respiratory distress syndrome−ARDS), and recovery [8,9], Fig. 2. This natural timing should be used to establish key point-of-care sampling timepoints, avoiding patient discomfort and to focus resources. Infection can progress or be aborted at every stage, presenting a dynamic continuum, to which macrophages and monocytes contribute, together with other immune and non-immune elements; MPS contributions to COVID-19 resistance and pathogenesis are still underestimated and poorly understood.

3.2. Co-morbidities and the mps

Macrophage activities contribute to many co- morbidities [8,9], including aging [28], obesity [29], diabetes [30], exposure to pollutants [31], and microbiome properties [32]. Macrophage functions alter with age, together with adaptive immune processes such as thymic involution, which deplete T lymphocyte reserves. Chronic, low grade inflammation (*INFLAMMAGING*) has been proposed as a predisposing factor [33]. This may result from failure of macrophages and NK cells to clear increasing accumulation of senescent cells, that enhance basal levels of inflammation and interfere with subsequent adaptive immunity upon infection [28]. Baseline inflammation may not be detrimental in itself, but can initiate an inflammatory cascade that amplifies excessive inflammation occurring in response to pathogens. Age-related metabolic correlates of macrophage

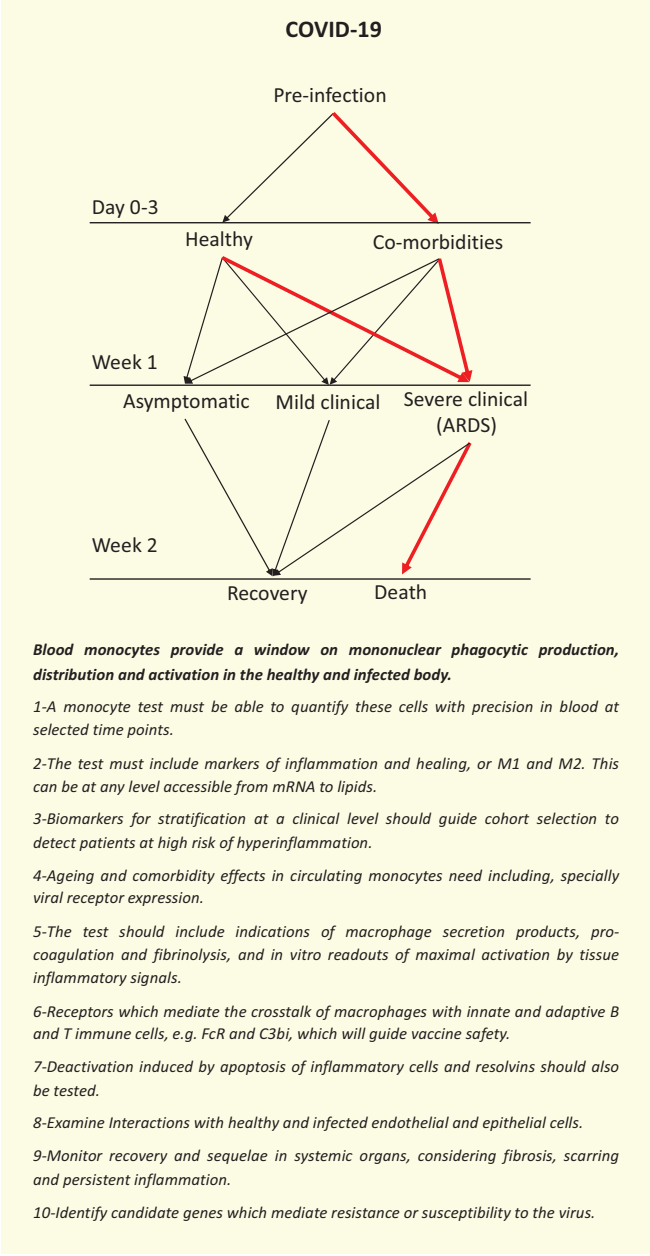

Fig. 2. Decision points that determine the outcome of COVID-19 infection and requirements for a monocyte activation assay. A time and severity pattern has emerged for COVID-19 progression. The first days, first and second week timelines are turning points for resolution or deterioration. Epidemiologic precision for these milestones and precise parameters will be valuable for strategic sampling and patient stratification, sparing resources and patient material. With limited volumes of peripheral blood, a window on progression of the infection can be obtained by studying activation of the mononuclear phagocyte system, largely represented by monocytes in blood. The cells are biosynthetically active and relatively long lived, ideal to trace long term effects of comorbidities and infection. We highlight selected functional categories that should be addressed in monocytes and can be adapted to particular applications.

dysfunction include innate lymphoid cell interactions with adipose tissue and associated macrophages, as well as inflammasome activation [34,35]. This affects thermogenesis, part of a wider metabolic syndrome involving glucose and lipid metabolism [36], elevated body mass index (BMI), type 1 and type 2 diabetes, obesity and atherosclerosis [37]. These correlate with macrophage metabolic and mitochondrial activities [38] and, possibly, ACE2 expression and hypertension, another co-morbidity [37]. ACE2, the COVID-19 entry receptor, is widely expressed on microvascular endothelium and binds the SARS-CoV2 spike glycoprotein implicated in angiotensin regulation by its competition with ACE1, and potential role as an anti-inflammatory agent [39]. Macrophages in tuberculosis and sarcoidosis express ACE1 [40], but expression of ACE2 by monocytes and tissue macrophages has not been validated [41]; evidence for productive infection of macrophages via ACE2 is incomplete [15]. Macrophages express scavenger receptors including Axl and MERTK, tyrosine kinase receptors implicated in phosphatidyl serine recognition and phagocytosis of apoptotic cells [21]; Axl is a coreceptor for ACE2+ dependent infection of other cell types by COVID-19 and other enveloped viruses via surface or endocytic routes [42], the basis for a clinical trial involving an Axl inhibitor.

Alveolar macrophages [43] are close to Type 1 pneumocytes, involved in gas exchange, Type 2 surfactant-producing pneumocytes, and capillary endothelium, all ACE 2+ [43]. They take up and store atmospheric pollutants and poorly-degradable pro-inflammatory particles; host DNA from dying cells can induce the innate STING pathway after uptake and also activate inflammasomes in lung macrophages after exposure to ozone and cigarette smoke [31]. Other airway predisposing conditions involving macrophages [8,9], include genetic disorders such as fibrocystic disease and structural abnormalities associated with chronic inflammation and infection.

Chronic infections in which TH1-activated macrophages play a major role, tuberculosis and AIDS, enhance the risk of severe infection, with odds ratios of 1.7 and 2.3– respectively. TH2-dependent asthma may not enhance COVID-19 susceptibility, possibly correlated with low levels of ACE2 expression [44]. IL-13 also reduces ACE2 expression, suggesting that M2-macrophages may contribute to viral resistance. BCG and other immunisations may transiently promote non-specific resistance to SARS-CoV2 [45], to be confirmed in trials. Mononuclear phagocytes are implicated in selected genetic [46] or acquired systemic immunodeficiency, immunosuppression and autoimmunity, including chronic renal disease requiring organ transplantation. Tumor associated macrophages (TAM) and Myeloid derived suppressor cells (MDSC) promote malignancy and metastasis, and with chemo-and radiotherapy may enhance COVID-19 risk. Microglia contribute to neurodegeneration through interaction with neurons in Alzheimer disease, a comorbidity independent of age.

Comorbidities are likely to be multifactorial, and not all display an obvious macrophage link; for example, the ACE2 locus on the X chromosome [47] may contribute to the decreased risk of females [48], but genetic and environmental factors underlying gender, racial and individual disparities remain unclear. Therapeutics for many comorbidities impact on macrophage inflammatory and metabolic functions, including statins, sugar stabilizers, anti-hypertensives and anti-inflammatory agents; control of diabetes and obesity are important in preventing severe infection. Glucocorticoids, which have potent anti-inflammatory effects on macrophages, are contra-indicated in early infection, while protecting against severe disease; timing of administration is therefore critical [49]. The use of anti-TNF antibody in treatment of rheumatoid arthritis, protects against subsequent severe COVID-19 requiring hospitalization [50].

In conclusion, the present evidence for a common macrophage contribution to a range of comorbidities is correlative, rather than causal. Nor is it clear whether they increase the risk as well as the severity of infection. Macrophages are central to inflammation, immunity and metabolic diseases, but further genetic and cellular studies are needed to provide a unifying hypothesis.

3.3. Asymptomatic and initial, mild infection

There is little direct evidence how monocytes and tissue macrophages contribute to asymptomatic infection, but their tissue distribution, receptor repertoire and secretory capacity are vital to viral resistance as well as dissemination. Acute macrophage antiviral and inflammatory responses determine the outcome of infection, together with other innate and adaptive host mechanisms. We

outline evidence from clinical [20] and experimental observations from earlier [51] and current [20] coronavirus studies and consider the role of macrophages and monocytes in local and systemic infections, the antiviral and inflammatory response, resolution and complications.

Most COVID-19 infections follow droplet inhalation and upper respiratory airway infection, which can be asymptomatic or mild, depending on mucosal immunity and IgA protection. Nasopharyngeal epithelium expresses ACE2 and is an early target for infection. Even if local tissue macrophages do not express ACE2 receptors and are not directly infected, they play a role in inflammatory cytokine responses to infected cells through surface, endosomal and cytosolic receptors and secretion of IL-1 family members [52] and antiviral interferons (IFNs). Monocytes, dendritic cells and tissue macrophages can bind virus through lectin-like receptors such as CD169 [53], for transport to regional lymph nodes. Delivery of virus droplets to the lower respiratory tract leads to infection of ACE2+ alveolar epithelium and capillary endothelium. Detection of intracellular RNA in alveolar macrophages could result from uptake of infected epithelial and endothelial cell debris rather than active infection. Single cell mRNA studies of bronchoalveolar lavage in COVID-19, have confirmed the recruitment of inflammatory monocytes as well as the presence of reactive alveolar macrophages in lung [17,18].

Several common symptoms of early infection can be the indirect result of epithelial cell necrosis by viral infection mediated by ACE2 and/or other co-receptors, such as transmembrane protease serine 2, (TMPRSS2) [13]. Uptake of virus or epithelial cell debris by lung macrophages can initiate inflammasome activation by P2RX7 and release IL-1β, Type1 IFN, IL-6 and TNF [3], contributing to fever, pain, lethargy and headache. Raised levels of C-reactive protein (CRP), induced in hepatocytes by IL-6, are acute phase, plasma biomarkers of COVID-19. Other macrophage products include chemokines such as IL-8 (CXCL8) and MCP-1 (CCL2), recruiting abundant PMN and monocytes to sites of infection. Through viraemia COVID-19 reaches systemic organs such as heart, gut, brain and kidney, which contain resident and recruited macrophage populations; these contribute to local, specialised dysfunctions, such as myocarditis and cardiac arrhythmia, following interactions of macrophages and infected cardiomyocytes [54]. Intravascular coagulation [3,24] promotes dissemination of microthrombi-emboli to lungs, heart and brain, and fibrin degradation D-dimers appear in blood. This is evidence of early resolution, enhanced by the generation of antiviral IgM and IgG.

We have little understanding of the innate and adaptive mechanisms that fail to clear local viral infection and at what stage IFN [55,56], other pro-inflammatory [57] and haematopoietic responses become dysregulated in blood monocytes [58]. Together with compromised lung oxygenation, this can lead to systemic immune and vascular dysfunctions. A syndrome of mild infection in younger subjects gives rise to chilblain-like inflammation in toes and fingers [59]. The uncommon multisystem Kawasaki-like inflammatory syndrome of children results from atypical immune responses to COVID-19 [60,61]. This involves anti-viral antibody, systemic vasculitis affecting heart rather than lung, and can respond to corticosteroids. Apart from platelet activation, mononuclear phagocytes are known to produce tissue factor and other procoagulants [62], Factors 5 and 13; fibrinolysis by vascular endothelium and inflammatory macrophages [63] is mediated by secretion of urokinase, which cleaves plasminogen to plasmin; its activity is regulated by inhibitors in plasma, such as alpha2 macroglobulin. Anticoagulants and intranasal or inhaled Type1 IFN may be useful inhibitors of thrombosis and local viral infection, limiting progression to more severe infection.

3.4. Moderate and severe infection: the hyperinflammatory syndrome

Between 8–12 days after COVID-19 infection, patients can progress to severe lung infection characterized by pneumonia, increased vascular permeability, edema, and hypoxia, requiring oxygen or mechanical ventilation. This is part of a systemic viral infection of ACE2+epithelia and endothelia, widespread cell death, ischaemia, hypotension and organ failure. Blood analysis at time of hospitalization, has revealed polymorphonuclear leukocytosis, altered levels of abnormal monocytes and lymphopaenia [58,64]. Myeloid cell recruitment to lung contributes to the hyperinflammatory and coagulopathy acute respiratory distress syndrome (ARDS) [20]. This is accompanied by monocyte/macrophage dysregulated production of secretory products. It is not clear which factors contribute to this complication: enhanced viral growth–perhaps triggered by early, poorly neutralizing IgM and complement or IgG, hypoxia and loss of antiviral mechanisms of macrophages–directly or secondary to lymphopaenia, massive local epithelial and endothelial necrosis, hyperactivation of newly recruited immature monocytes, or a “perfect storm” encompassing several of the above. This syndrome could be triggered by induction of ACE2 on specific populations of monocytes and macrophages, resulting in their direct infection. By this stage, spleen and draining nodes at sites of infection are severely disorganized [65]. M1-like monocytes carrying virus through CD169 or other receptors, whether infected or not, can disseminate the virus throughout the body by a Trojan horse mechanism [66]. It is not known whether lymphocytes, not infected, die by apoptosis or necrosis, from products of activated lymphocytes or macrophages such as TNF, or by phagoptosis. We summarize evidence that blood monocytes and activated tissue macrophages contribute to severe COVID-19 and consider virus-induced macrophage pathogenetic mechanisms, which can be targeted therapeutically.

Earlier studies on SARS1/MERS revealed many of the features of ARDS and of macrophage involvement [6,51]. Initial blood and tissue analysis of COVID-19 provided morphologic evidence of monocyte abnormality and intense macrophage phagocytic activity in infected organs [65]. FACS and single cell RNA analysis of monocytes and broncho-alveolar macrophages [18] provided evidence of emergency myelopoiesis [64] and confirmed the presence of recruited and activated macrophages in alveoli [17] and lung interstitium. Plasma membrane opsonic and other receptors contribute to dysregulated inflammation, monocytic hyperactivation and impaired phagocytic clearance of apoptotic and necrotic cells and debris. Acting through NFkB, RNA, DNA and other sensing and signaling pathways, they initiate production of numerous secretory products. Particular antiviral and proinflammatory products have been targeted for anti-inflammatory treatment, including the IFNs [55,56], IL-1 family [52], IL-6 [27] and TNF [22]. In addition, mononuclear phagocytes interact with plasma cascades, and through upregulated adhesion molecules, with other myeloid and lymphoid cells, platelets, system-wide endothelia and epithelia.

Dysregulated activation of macrophages contributes to the hyperinflammatory and thrombotic pathways of severe COVID-19 [3,24,67].Virus-induced cell injury, apoptosis, necrosis and necroptosis are sensed by distinct, opposing macrophage receptor-dependent effector responses. These include activating and inhibitory pathways of IL-1 production, processing and secretion [52,57], triggered by tissue injury, inflammasome and caspase activation. A highly impaired type1 IFN response, characterized by no IFNβ and low IFNα production and activity, has been associated with persistent viraemia and an exacerbated inflammatory response, partially driven by NFkB, TNF and IL-6 [56]. Endogenous oxidized phospholipids reprogramme macrophage metabolism and boost hyperinflammation [68]. By capturing inflammatory lipids released from dying cells, CD14 induces inflammasome-dependent phagocyte hyperactivation [69]. Macrophage fuel production and utilization [36] are sensitive to hypoxia, HIF-1 responses [70] and iron availability, influencing M1 polarization and effector functions. Activated macrophages trigger coagulation and complement cascades, as well as angiotensin and bradykinin pathways that dysregulate vascular tone and permeability, exacerbating inflammation and edema.

Immunological processes that may contribute to the outcome of macrophage- viral induced pathology include antibody enhancement of infection (ADE) and inflammation (ADI), and neutrophil NETosis. Anti-spike antiserum can induce hyperinflammation via macrophage FcR, depending on IgG glycosylation and Syk involvement [71]. Cross-reacting antibodies from previous benign coronavirus infections also contribute to enhanced pathology [72]. Dendritic cell dysfunction, ascribed to down regulated HLA-DR expression [67,72] and defective antigen presentation to T and B lymphocytes, may result from lack of costimulatory signals, aborting lymphocyte proliferation and activation and mediating widespread clonal death. Haemophagocytosis, a feature of the macrophage activation syndrome (MAS), is also observed in other viral infections.

3.5. Recovery

Macrophages are central to the cellular processes which contribute to recovery from COVID-19 infection; they remove debris and necrotic tissue through phagocytosis and secretion of elastase and collagenase, and produce IGF1 and TGF-β to promote tissue recovery. M2-like macrophages are the source of potent lipid metabolites which resolve inflammation; these include resolvins, protectins and maresins, short lived autacoids which regulate the magnitude and duration of acute inflammation [25,73]. Acting through macrophage GPCRs for these metabolites, they enhance efferocytosis of dying neutrophils, and counter inflammation without immunosuppression.

Recovery can be prolonged after severe infection, involving fever, lethargy, scarring and loss of organ function. Italiani and colleagues profiled the course of resolving versus persistent inflammation with special reference to IL-1 family molecules [57]. An independent single cell mRNA analysis of peripheral blood mononuclear cells during early recovery of COVID-19 infection [23], revealed an increased ratio and level of classical CD14^{++}IL-1β+ monocytes. Further studies are needed to compare monocytes and tissue macrophages after mild or severe infection and to establish whether repolarisation of macrophages from M1 to an M2 phenotype can promote resolution and recovery after COVID-19. After helminth infection, M2 macrophages express resistin, which protects against LPS-induced toxic shock [74]. New or repurposed therapies may be able to modify the sequelae of severe infection.

4. Monocyte activation Assay: rationale

Blood monocytes provide a window on mononuclear phagocyte production, distribution and activation in the healthy and infected body. The level of circulating monocytes depends on circadian release from bone marrow, adhesion to endothelial surfaces and tissue entry. Blood from hospitalized patients has shown a decrease in the proportion of monocytes, increased size, low CD14, high CD169 and low HLA-DR consistent with release of immature, more replicating Ki67+ monocytes from the bone marrow during emergency haematopoiesis [64]. Monocytes respond through specific plasma membrane receptors to CSF1, CSF2 and CSF3 during differentiation, and to IFNs, pro- and anti-inflammatory cytokines, during immune activation. In the steady state, monocytes circulate with a half-life of 1–2 days; turnover is enhanced after LPS [75] and severe influenza infection, which induces morphologic changes and a pro-inflammatory M1-like activation phenotype [4], similar to changes in COVID-19 [64]. This provides a rationale for use of monocyte gene expression and antigen markers, combined with in vitro exposure to phagocytic and TLR stimuli, to assess maximal pro-inflammatory M1 activation. The levels of TNF and other proinflammatory cytokines observed in vivo during COVID-19 may be lower than after bacterial sepsis. It is therefore essential to extend analysis to a broad range of secretory products.

4.1. Considerations

The balance of pro- versus anti-inflammatory activation determines the protective versus deleterious impact on the host. In vivo priming, combined with innate and adaptive immune triggers in vitro, provide a surrogate assay for potential activation of monocyte-derived macrophages in tissues, Fig. 1. The number of monocytes and their activation status can be assayed by combining analysis of CSF1-, CSF2- and CSF3- receptors during growth and differentiation, with markers of M1/M2-like activation; gene expression biomarkers are available at different stages of COVID-19 infection for each pathway, and in response to a range of activating and deactivating stimuli. Fig. 2 illustrates testing points in the natural history of COVID-19 infection and lists requirements for a comprehensive monocyte activation assay. This provides insights into the contribution of monocytes to pathogenesis, and guides treatment. Monocyte tests can be combined with viral and antibody tests at any stage of infection, providing access to an intermediate stage between haematopoiesis and tissue effector functions. Regulators of monocyte activation which can be tested in vitro include innate and adaptive immune cells, viral proteins, antiviral agents and anti-macrophage therapeutics.

Reconstitution of lymphopaenia may contribute to a viral-induced storm resembling the Immune Reconstitution Inflammatory Syndrome (IRIS) observed in tuberculosis patients with AIDS as lymphocyte counts recover during anti-retroviral treatment [76]. The phenomenon of antibody-dependent enhancement (ADE) of flavivirus infections [10], has not been demonstrated for COVID-19, to date. However, anti COVID-19 antibodies and immune complexes, induced by prior vaccination, during the course of the disease or by passive transfer of IVIg or pools of “elite” mab [5,71], may enhance dysregulated inflammatory responses of primed monocytes through Fc- and complement receptors. This entails characterization of ITAM/ITIM monocyte FcR functions, analysis of reactive oxygen and nitrogen metabolites, cytokine secretion, procoagulant and fibrinolytic responses, and cellular cytotoxicity. Longitudinal studies of monocyte activation and cost/benefit analysis of any immune interventions are therefore mandatory.

5. PERSPECTIVE

What distinguishes SARS-CoV2 from other viruses, including other coronaviruses, although the hyperinflammatory storm is clearly a feature of its forerunners [6]. A strong candidate must be the expression level of ACE2 as a viral entry receptor. The host also determines the outcome of exposure to the virus. In his prescient views of the role of the external and internal host environment, Rene Dubos differed from the standard germ theory of infectious disease, as necessary, but not sufficient [77]. The pre- and post- clinical features of the present pandemic illustrate this concept superbly. Although host susceptibility and severity of infection are clearly multifactorial, there is a thread from the role of its receptor in angiotensin regulation, vascular tone and permeability, through hypertension and cardiovascular function. The connection with the metabolic syndrome suggests an unknown link to lipid metabolism, including interactions of adipocytes and macrophages [35].

Separate streams of the mononuclear phagocyte lineage co-exist to generate monocytic, granulocytic and dendritic cell-like monocytes [78], and the impact of COVID-19 infection on monocyte activation needs to be determined to identify targets for diagnosis and intervention. The different monocytic lineage receptors for CSF1, 2 and 3 provide direct markers of disturbed haematopoiesis and skewed cell differentiation [79]; and can be combined with downstream effectors such as CD14 [80] and FcR to determine their full activation potential. Anti-growth factor/cytokine receptor antibodies may therefore be more effective in attenuating a hyperinflammatory storm and attendant organ damage than using individual anti-

cytokine reagents for GM-CSF, IL-6 and TNF, for example [22]. Recent studies with anti CSF2R blockade by mavrilimumab show promise [12,81] and anti CSF1R blockade presents an alternative or additional option. Repurposing trials of anti-inflammatory drugs have targeted a variety of macrophage pathways, with mixed results. In each case, we have to weigh potential benefits against interfering with macrophage antimicrobial and other essential functions such as anti-viral-antibody dependent killing, clearance of necrotic cells and debris, and repair, as well as the risk of potentiating inflammatory responses of mononuclear phagocytes through antibody -dependent mechanisms.

Macrophage "activation" is a complex process with limited knowledge of its regulation or variable expression in different tissues and by diverse causes [7]. Reciprocal interactions between mononuclear phagocytes and adaptive T and B lymphocyte subtypes play a decisive role in the control of macrophage activation. Further studies on monocytes from infected patients will establish how this virus manipulates innate and adaptive control mechanisms to facilitate its survival and spread [82,83].

6. OUTSTANDING questions

1. Define the molecular basis for mononuclear phagocyte contributions to different stages of COVID-19 resistance and pathogenesis.
2. Define interactions between mononuclear phagocytes, COVID-19 and other host cells in different tissues.
3. Develop tests to quantify biomarkers of monocytes and tissue macrophages during COVID-19, for diagnosis, prognosis, therapy and prevention.

Contributors

The review was conceived and written by SG and FOM. TC and FO contributed experimental findings and artwork. All authors have read and approved the final version of the manuscript.

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Search strategy and selection criteria

We have used PUBMED and searched a range of journals for mostly recent refereed papers dealing with mononuclear phagocytes and SARS2 COVID-19 and attended a range of webinars organised by journals, Immunological Societies and Universities.

Declaration of Competing Interest

SG is a consultant to Verseau and to Myeloid Therapeutics, both exploiting Tumour associated macrophages for potential cancer immunotherapy. Other authors declare no conflicts of interest.

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