Review article

From rare to more common: The emerging role of omics in improving understanding and treatment of severe inflammatory and hyperinflammatory conditions

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Inflammation is a pathogenic driver of many diseases, including atherosclerosis and rheumatoid arthritis. Hyperinflammation can be seen as any inflammatory response that is deleterious to the host, regardless of cause. In medicine, hyperinflammation is defined as severe, deleterious, and fluctuating systemic or local inflammation with presence of a cytokine storm. It has been associated with rare autoinflammatory disorders. However, advances in omics technologies, including genomics, proteomics, and metabolomics, have revealed it to be more common, occurring in sepsis and severe coronavirus disease 2019. With a focus on proteomics, this review highlights the key role of omics in this shift. Through an exploration of research, we present how omics technologies have contributed to improved diagnostics, prognostics, and targeted therapeutics in the field of hyperinflammation. We also discuss the integration of advanced technologies, multiomics approaches, and artificial intelligence in analyzing complex datasets to develop targeted therapies, and we address their potential for revolutionizing the clinical aspects of hyperinflammation. We emphasize personalized medicine approaches for effective treatments and outline challenges, including the need for standardized methodologies, robust bioinformatics tools, and ethical considerations regarding data privacy. This review aims to provide a comprehensive overview of the molecular mechanisms underpinning hyperinflammation and underscores the potential of omics technologies in enabling successful clinical management. (J Allergy Clin Immunol 2025;■■■:■■■-■■■.)

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Severe inflammation and hyperinflammation associated with disease occur when the normally beneficial activation of inflammatory signaling pathways (Fig 1) and resulting inflammatory responses exceeds in severity and duration, thereby causing tissue damage and ultimately harm to the host. *Hyperinflammation* is classically defined as systemic severe inflammation with cytokine storm; the term likely entered English medical terminology in 1994.[1] Since then, hyperinflammation has largely been used to describe overzealous cytokine release by innate, myeloid lineage immune cells, mainly of polymorphonuclear and later of monocytic lineages.

Innate inflammatory cytokines attempt to contain pathogens and other potentially damaging events. Until they are activated, they are contained by specific adaptive immune responses or processes like programmed cell death to minimize damage to the host. Consequently, severe inflammation can occur in a wide range of manifestations. In more common (polygenic) diseases, local detrimental inflammation is seen, for example, in gout, arthritis and periodontitis, but systemic hyperinflammation is evident in secondary hemophagocytic lymphohistiocytosis (HLH). In various monogenic inborn errors of immunity (IEI), hyperinflammation or severe local inflammation may be seen in such disorders as autoinflammatory necrotizing fasciitis, familial HLH limited to the central nervous system, keratitis fugax hereditaria,[2] and chronic granulomatous disease.

Hyperinflammation seems to occur not only in severe and complicated HLH and inflammasomopathies but also in proteasomopathies, type I and type II interferonopathies, and less easily classified monogenic autoinflammatory diseases. The diversity and complexity of clinical manifestations among patients with hyperinflammatory syndromes continue to expand with the identification of new genetic and immunotherapeutic triggers. This underscores the importance of personalized approaches in managing these conditions, even in polygenic traits with described deep-intronic genome-wide association study associations.[3]

Hyperinflammation may be incited by various, often multiple, internal or external factors. Internal factors include sensing of

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Abbreviations used

AI: Artificial intelligence
AP-MS: Affinity purification–MS
CK2: Protein kinase 2
COVID-19: Coronavirus disease 2019
CVID: Common variable immunodeficiency
DIA: Data-independent acquisition
HLH: Hemophagocytic lymphohistiocytosis
IEI: Inborn errors of immunity
iTRAQ: Isobaric tags for relative and absolute quantitation
JAK: Janus kinase
MAPK: Mitogen-activated protein kinase
MAS: Macrophage activation syndrome
MIS-C: Multisystem inflammatory syndrome in children
MS: Mass spectrometry
NF-κB: Nuclear factor kappa–light-chain enhancer of activated B cells
NK: Natural killer
NLRC4: NLR family CARD domain–containing 4
NLRP3: Nod-, LRR-, and pyrin domain–containing protein 3
PBMC: Peripheral blood mononuclear cell
PEA: Proximity extension assay
PRF1: Perforin 1
SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2
sJIA: Systemic juvenile idiopathic arthritis

danger signals by pattern-recognition receptors, monogenic inheritance, polygenic inheritance, and somatic mutations, and external factors include sensing of microbial patterns in viremia, bacteremic sepsis, and secondary HLH. Modifying factors further include detrimental host immune responses of secondary and/or primary immunity due to autoimmunity (horror autotoxicus[4]) or autoinflammation (horror autoinflammaticus[5]), respectively. Whatever the causes, for effective therapeutic approaches, we need to comprehensively decipher the cellular mechanisms resulting in excessive cytokine production in each form of hyperinflammation. An example of the complexities involved are multimorphic IEIs causing hyperinflammation in the host, where a single gene mutation may result in concomitant hypomorphic, hypermorphic, and neomorphic immune reactions. However, few technologies have the capacity to decode such complex mechanisms.[6,7]

Recent advancements in omics technologies have allowed researchers to delve deeper into the complex mechanisms underpinning hyperinflammatory responses. This has led to hyperinflammation being increasingly recognized as a key player in a variety of severe and critical illnesses, including coronavirus disease 2019 (COVID-19). Specifically, bottom-up proteomics, a prevalent strategy in mass spectrometry (MS)-based proteomics, has been instrumental in identifying new targets for therapeutic intervention and in shaping personalized medicine by dissecting the proteome into digestible peptides for in-depth analysis. Omics technologies encompass a broad range of approaches, such as genomics, transcriptomics, proteomics, and metabolomics, that offer comprehensive and high-resolution insights into biological systems. Furthermore, the growing field of personalized medicine presents opportunities to leverage omics technologies to devise targeted treatments for hyperinflammation, thereby transforming a once-rare condition into a common target of therapeutic interventions.[3] This review will discuss the role of omics technologies, with a significant emphasis on bottom-up proteomics, in advancing our understanding of hyperinflammation and improving targeted treatments.

GENOMICS OF HYPERINFLAMMATION

The genetic basis of hyperinflammation is complex and multifactorial, involving interactions between numerous genes and environmental factors. Genetic predispositions play a substantial role, with mutations in specific genes driving an overactive immune response that manifests in a wide range of inflammatory diseases that sometimes include a hyperinflammatory component to differing degrees of severity. Whole-genome sequencing has emerged as a powerful tool for elucidating the genetic underpinnings of hyperinflammation across a spectrum of diseases. By providing a comprehensive overview of the entire genome, whole-genome sequencing allows for the identification of genetic variations, such as single nucleotide variations, copy number variations, and structural variants, that drive pathologic conditions.[8-10] This approach has been instrumental in uncovering specific genetic markers and mutations associated with heightened inflammatory responses.[11,12] However, it is important to note that certain genetic mechanisms, such as complex structural variants or repetitive regions, may not be fully captured by short-read sequencing technologies, indicating the need for continued technological improvements.[3]

Hyperinflammation is frequently encountered within the IEI categories defined by the International Union of Immunological Society Expert Committee, particularly in "Diseases of Immune Dysregulation and Autoinflammatory Disorders," as well as in nonheritable "Phenocopies of IEI." These conditions exhibit pronounced variability between individuals, genes, and gene mutations, as exemplified by Nod-, LRR-, and pyrin domain–containing protein 3 (NLRP3)- and NLR family CARD domain–containing 4 (NLRC4)-associated diseases, which demonstrate highly variable phenotype severity across different cases.[2,13] Similarly, the frequency of hyperinflammatory episodes varies significantly across these disorders. For instance, mutations in *MEFV* are associated with familial Mediterranean fever, a rare autoinflammatory disease characterized by recurrent episodes of fever and inflammation caused by mutations affecting pyrin, a protein that regulates inflammatory responses.[14] While true hyperinflammatory crises resembling HLH are relatively rare,[15] the discovery of *MEFV* mutations has expanded our understanding of inflammasomopathies, which now encompass a growing group of disorders caused by mutations in various inflammasome components.

Another seminal example of how genetic insights can guide our understanding of hyperinflammatory mechanisms is the identification of gain-of-function variants in the *NLRC4* gene. *NLRC4* encodes a key component of the inflammasome, which is a protein complex that, when activated, promotes the maturation and secretion of proinflammatory cytokines, notably IL-1β and IL-18. Mutations in *NLRC4* have been shown to cause a spectrum of autoinflammatory disorders, often resulting in fevers, enterocolitis, and, in severe cases, an HLH-like hyperinflammatory phenotype.[13,16] By pinpointing the precise genetic lesion underlying such conditions, these variants have illuminated the critical role of uncontrolled inflammasome activation in driving systemic hyperinflammation.

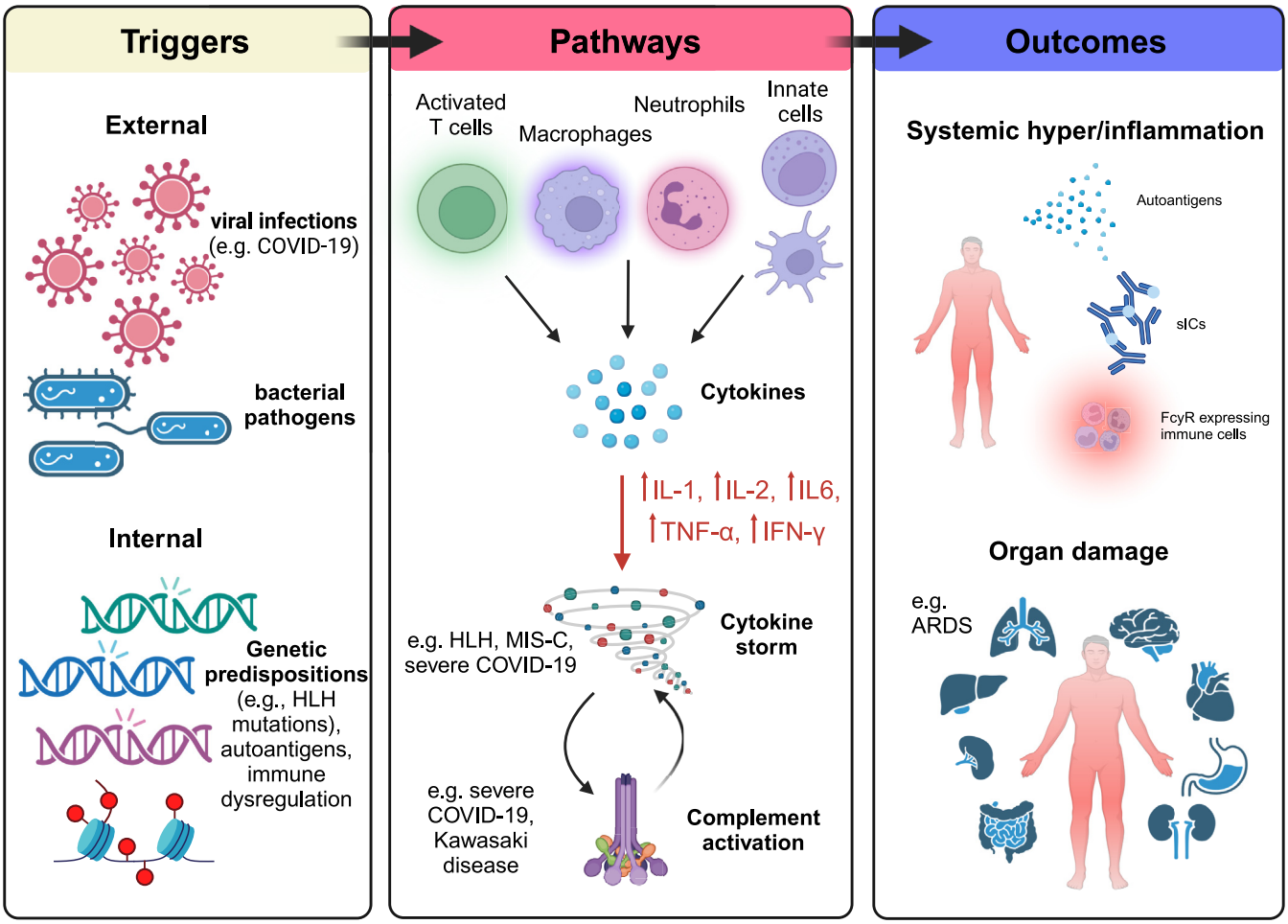

FIG 1. Pathways leading to hyperinflammation and key mechanistic axes. Schematic highlight of both external triggers (eg, viral infections like SARS-CoV-2, bacterial infections) and internal factors (eg, genetic predispositions like familial HLH mutations, autoantigens, immune dysregulation) that converge to drive hyperinflammation. Once activated, several immune pathways, including cytokine overproduction (IL-1, IL-2, IL-6, TNF-α, IFN-γ), macrophage hyperactivation, neutrophil recruitment, and complement activation, lead to exaggerated inflammatory response. Resultant cascade can cause systemic hyperinflammation, multiple organ damage, and acute respiratory distress syndrome.

Further, they have provided a blueprint for targeted therapies. For instance, blocking IL-1 activity has long been considered a viable strategy in autoinflammation, but certain *NLRC4*-driven hyperinflammatory syndromes respond more effectively to IL-18 inhibition.[17] Thus, studies of *NLRC4* variants not only reinforce the centrality of inflammasome dysregulation in hyperinflammation but also demonstrate that genetic data can directly inform rational therapeutic choices, highlighting IL-1 and IL-18 antagonism as actionable interventions to dampen excessive inflammatory cascades. These insights underscore how genomics can bridge mechanistic understanding and clinically meaningful, personalized therapies in hyperinflammatory conditions.

While adequate amplification guides inflammation and hyperinflammation, well-functioning microbial sensing is also important. For example, NOD2 is an intracellular microbial sensor of the innate immune system that is essential for maintaining immune homeostasis.[18] *NOD2* mutations are linked to Crohn disease, autoinflammatory granulomatous diseases such as Blau syndrome, and early-onset sarcoidosis.[19,20] Appropriate control of overzealous inflammation is further dependent on negative feedback. A20 is crucial for the negative feedback regulation of nuclear factor kappa–light-chain enhancer of activated B cells (NF-κB) signaling. Mutations in the *TNFAIP3* gene, which encodes the protein A20, are associated with early-onset autoinflammatory and autoimmune syndromes, systemic inflammation, and conditions like rheumatoid arthritis.[21-23]

STAT3 loss-of-function mutations are connected to hyper-IgE syndrome, a rare primary immunodeficiency characterized by chronic lung and skin infections and inflammation as well as involvement of soft and bony tissues. These mutations impair both innate and adaptive immune responses, leading to elevated serum IgE and eosinophilia,[24-26] which are hallmarks of inflammation. Conversely, gain-of-function mutations in *STAT3* result in a complex condition marked by variably severe autoinflammation and autoimmunity,[27,28] reflecting the diverse roles of STAT3 in immune regulation.

Further emphasizing the intricate effects of genetic mutations on hyperinflammation, a study of a *STAT1* gain-of-function

variant shed light on the genetic underpinnings of infection susceptibility and hyperinflammation.[29] In this case, a child from consanguineous parents exhibited severe infections leading to HLH and liver failure. The discovery of a homozygous *STAT1* gain-of-function variant revealed increased STAT1 activity, resulting in the overproduction of interferon-stimulated genes. This hyperactivation of the immune response not only heightened the child's susceptibility to viral infections but also contributed to a hyperinflammatory state, underscoring the critical role of STAT1 in immune regulation.

Genetic susceptibility to inflammation is also influenced by polymorphisms in cytokine genes that modulate the expression levels, intensity, and fine-tuning of inflammatory responses. Specifically, variations in genes encoding IL-1β, IL-6, and TNF-α have been associated with an increased risk of developing Alzheimer disease and chronic venous disease, which both have inflammatory components.[30,31] For Alzheimer disease, individuals with specific IL-10, IL-6, and TNF-α polymorphisms were more likely to develop pathologies associated with Alzheimer disease, which may be a consequence of increased neuroinflammation.[30] Inflammation is also a hallmark in chronic venous disease, causing endothelial dysfunction. Polymorphisms in anti-inflammatory cytokine genes have also been associated with increased risk of complications during pregnancy due to premature rupture of membranes.[32]

In the context of infectious diseases like dengue, genetic variations in natural killer (NK) cell functional genes, specifically *KLRK1* and *PRF1* (perforin 1), have been associated with a hyperinflammatory response and more severe disease outcomes. A study revealed that features of hyperinflammation, including higher biomarkers and impaired NK cell function, are associated with dengue severity, and that polymorphisms in NK cell cytolytic function genes (*KLRK1* and *PRF1*) play a role in this hyperinflammatory response.[33] Rare *PRF1* variants are also associated with secondary HLH and hyperinflammation after severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.[34] Moreover, mutations in the *PRF1* gene, encoding the pore-forming protein perforin, have been associated with familial type 2 HLH, which is a rare but severe disorder characterized by life-threatening hyperinflammation caused by a massive release of cytokines. These studies highlight the key role of perforin in immune system activation and inflammation.[35]

Life-threatening hyperinflammation also frequently occurs in systemic juvenile idiopathic arthritis (sJIA) and adult-onset Still disease, and rare HLH variants are enriched in sJIA patients.[36] sJIA and adult-onset Still disease patients frequently present with episodes of macrophage activation syndrome (MAS), a hyperinflammatory complication that is clinically and mechanistically similar to HLH.[37,38] Estimates suggest that overt MAS occurs in up to 10% to 20% of sJIA cases, and subclinical MAS may be even more frequent. This indicates that hyperinflammatory episodes are a comparatively common event in this patient population.[37,38] Primary HLH is typically linked to biallelic mutations in genes related to the perforin cytolytic pathway (eg, *PRF1, UNC13D, STXBP2*), and can predispose patients with sJIA to severe hyperinflammatory episodes.[39,40] This partial genetic overlap emphasizes that hyperinflammation can arise at the intersection between common inflammatory disorders and underlying genetic susceptibility factors. Thus, although the fundamental driver in sJIA/adult-onset Still disease may not be a single gene defect, subclinical genetic variations affecting cytotoxic pathways can tilt the balance toward uncontrolled inflammation. Identifying these variants may improve risk stratification and guide personalized therapeutic strategies, such as the targeting of IL-1, IL-18, or IFN-γ, which are central mediators in both HLH and MAS.

Kawasaki disease, another inflammatory condition, shares some immunologic and clinical features with sJIA, including systemic inflammation. However, hyperinflammatory responses akin to MAS or HLH are much rarer in Kawasaki disease.[41-43] Although both diseases involve dysregulated innate immunity and cytokine production, the genetic landscape and threshold for triggering severe hyperinflammation differ. Understanding these distinctions will help to refine diagnostic criteria and inform treatment strategies tailored to each condition's unique pathophysiology.

Despite some overlap, the distinctions between autoinflammatory and autoimmune disorders are crucial because they guide therapy. Autoinflammatory diseases are primarily innate immune disorders triggered by endogenous or exogenous factors, while autoimmune diseases involve abnormal adaptive immune responses to self-tissues. Further complex conditions, like systemic lupus erythematosus, exhibit characteristics of both, underscoring the complex interplay between innate and adaptive immunity in hyperinflammatory responses.[44]

Adding further complexity is the concurrent increase in discoveries of interferonopathies, which cause excessive production of type I or II interferons, and of mixed diseases leading to increased production of both interferons and inflammasome-associated cytokines. Furthermore, it has become increasingly clear that both somatic mutations and autoantibody-induced mechanisms can similarly cause hyperinflammation and IEI-like phenotypes.[13] For instance, mutations in the *UBA1* gene have been identified in patients with VEXAS syndrome, a rare but severe adult-onset autoinflammatory condition characterized by systemic inflammation and bone marrow dysplasia.[45,46] This underscores how acquired genetic changes can contribute to hyperinflammatory phenotypes mimicking IEI.

Currently, there are over a thousand human protein-coding genes associated with the keywords *inflammation* and *inflammatory* in the National Center for Biotechnology Information's gene database. Expanding the list to include their interactors results in a large set of genes/proteins involved in a variety of cellular functions. This further highlights the enormous complexity and diversity of inflammation.

While some variability in cytokine profiles and inflammatory mediators exists across different hyperinflammatory conditions, increasing evidence points to a core immunophenotypic and cytokine signature uniting these syndromes. Regardless of the initiating trigger or underlying etiology, primary HLH, secondary HLH, MAS, and severe manifestations of infections like COVID-19 often share a central axis of NK and $CD8^+$ T-cell overactivation, accompanied by elevated IL-18 and IFN-γ levels.[47-51] Although the relative prominence of these features may differ by disease context, this fundamental immunopathologic convergence suggests a common mechanistic framework. Recognizing and focusing on these core elements can improve diagnosis, guide disease monitoring, and inform targeted therapeutic strategies aimed at modulating these key drivers of severe inflammation.

PROTEOMICS AND HYPERINFLAMMATION

Proteomics is the large-scale study of proteins and is a critical branch of omics technologies. It enables the extensive mapping of the proteome: the entire set of proteins expressed by an organism or a specific tissue.[52] In the context of hyperinflammation, proteomics plays a pivotal role in deciphering complex protein networks and identifying biomarkers for diagnosis and therapy.[53,54] Here we delve the application of various proteomic technologies, including quantitative proteomics, phosphoproteomics, interaction proteomics, and single-cell proteomics, for identifying proteomic signatures of inflammation and hyperinflammation, and their roles in disease prognostics and prevention (Table I).

Proteomic signatures of hyperinflammation

Inflammation and hyperinflammation are characterized by marked alterations in the expression patterns of numerous inflammatory mediators including cytokines and chemokines.[55] In this context, quantitative proteomics emerges as an indispensable tool, providing a robust platform for the comprehensive and precise quantification of protein changes.[56] Techniques such as isobaric tags for relative and absolute quantitation (iTRAQ)[57] and tandem mass tags[58] have been instrumental in this endeavor. For example, iTRAQ, known for its ability to simultaneously compare the abundance of proteins across multiple samples, has been successfully used to investigate a spectrum of hyperinflammatory diseases, including sepsis[59-61] and acute respiratory distress syndrome.[62]

Several studies have utilized proteomic signatures to identify novel disease mechanisms in inflammation. For instance, proteomic pathway analysis focusing on the Fas pathway and caspase network proteins shed light on the role of inflammation in increasing the accumulation of myeloid-derived suppressor cells, revealing that inflammation may boost myeloid-derived suppressor cell accumulation by enhancing their resistance to Fas-mediated apoptosis.[63] In another study examining immune dysregulation in common variable immunodeficiency (CVID), targeted serum proteomics was used to stratify patients and identify key cytokine and chemokine signaling pathways. The study used the Olink proximity extension assay (PEA) technology to analyze a panel of 180 markers, identifying T_H1- and T_H17-associated signaling pathways, thereby highlighting the complexity of hyperinflammatory responses in CVID.[64]

The investigation of cytokine storms, which are severe systemic inflammatory responses characterized by excessive cytokine release, has significantly advanced through the application of proteomic methods. Techniques such as polyacrylamide gel electrophoresis, sodium dodecyl sulfate–polyacrylamide gel electrophoresis, high-performance liquid chromatography, MS, cytometric bead assays, and next-generation microarrays have been pivotal in elucidating the complex mechanisms driving these storms. Proteomics has enabled the identification of specific protein signatures and signaling pathways involved in several different disease states, providing insights into how cytokine dysregulation leads to multiorgan failure. These studies have deepened our understanding of the systemic hyperinflammation seen in conditions like severe COVID-19 and have contributed to the development of biomarkers used to diagnose and monitor severe inflammatory conditions (Table I). Moreover, these proteomic findings have facilitated the development of targeted therapeutic interventions, such as the use of cytokine inhibitors, which are currently being used to mitigate the effects of cytokine storms in clinical settings.[65]

Notably, recent quantitative proteomics (iTRAQ) studies have identified urinary protein markers linked to sepsis severity, revealing new insights into inflammatory processes,[61] while other iTRAQ-based serum proteomics analyses have found vimentin associated with sepsis severity and altered cytokine release.[60] Data-independent acquisition (DIA)-based serum proteomics in sepsis revealed additional biomarkers like ApoC3, VCAM1, and B2M.[59] Similarly, DIA-based plasma proteomics in COVID-19 identified severity-linked biomarkers guiding hyperinflammation management,[66] and Olink PEA-targeted proteomics in CVID highlighted IL-10, IL-12RB1, and CD83 signatures of chronic inflammation and immune exhaustion.[64] This growing body of research underscores the transformative impact of proteomics in improving both the diagnosis and treatment of hyperinflammation, making it an invaluable tool in modern medical research.

Interaction proteomics in hyperinflammation

Interaction proteomics is essential for mapping the complex networks of protein–protein interactions[67] that underlie cellular signaling and immune responses, including hyperinflammatory conditions. Understanding these interactions is crucial for elucidating the molecular mechanisms driving excessive inflammatory responses and identifying potential therapeutic targets.

One primary method used in interaction proteomics is affinity purification–MS (AP-MS). This technique uses a tagged protein of interest to pull down its interacting partners from cell lysates, followed by MS to identify the components of the complex. AP-MS has been pivotal in characterizing protein interactions involved in various signaling pathways that regulate inflammation such as the NLRP3 inflammasome, which is a multiprotein complex central to the activation of proinflammatory cytokines like IL-1β and IL-18.[68] Additional methods that capture more transient interactions[69] could be exploited to identify dynamic protein interactions relevant to inflammation. Capturing and analyzing these complexes provides invaluable insights into how specific protein interactions contribute to the regulation of inflammation and the potential dysregulation seen in hyperinflammatory conditions.

The application of these techniques in hyperinflammation research has led to significant discoveries regarding how genetic mutations can alter protein interaction networks and contribute to disease pathology. For instance, mutations in key inflammatory regulators such as *NFKB1* and *TNFAIP3* have been shown to disrupt their interaction networks, leading to enhanced or dysregulated inflammatory signaling. In one study, mutations in *NFKB1,* encoding a subunit of the NF-κB transcription factor, were found to disrupt its interactions, resulting in varied immunologic phenotypes and an increased risk of hyperinflammation.[70] Similarly, alterations in *TNFAIP3,* which encodes the anti-inflammatory protein A20, impaired its ability to interact with other proteins, enhancing NF-κB signaling and promoting inflammasome activation, which are key features of hyperinflammatory responses.[68]

Notably, a stop-gain mutation in *NFKB1* was found to cause hyperinflammatory responses and enhanced inflammasome activation.[70] More recently, the identification of truncating *NFKB1*

TABLE I. Proteomics studies providing methods and insights into hyperinflammation and inflammation

Study focus	Proteomics technique	Key findings	Reference (PubMed ID)
Quantitative proteomics			
Urinary proteomics in sepsis	Quantitative proteomics (iTRAQ)	Identified urinary protein markers linked to sepsis severity, offering insights into inflammatory processes.	23425763
Serum proteomics in sepsis/septic shock patients	Quantitative proteomics (iTRAQ)	Vimentin associated with severity; its elevation drives lymphocyte apoptosis and altered cytokine release.	30952998
Serum proteomics in sepsis	Quantitative proteomics (iTRAQ)	ApoC3, VCAM1, B2M, ApoE supplement classical markers (PCT, CRP) for improved sepsis diagnosis.	34825737
Complement activation in COVID-19	Quantitative proteomics (DIA)	Complement proteins linked to hyperinflammation; potential therapeutic targets identified.	34139154
Plasma proteomics in COVID-19 patients	Quantitative proteomics (DIA)	Biomarkers for severity identified, guiding strategies to manage hyperinflammation.	36812516
Serum proteomics in CVID	Targeted proteomics (Olink PEA)	IL-10, IL-12RB1, CD83 signature indicates chronic inflammation and potential immune exhaustion in CVID.	33190167
Phosphoproteomics			
LPS-stimulated macrophages	Quantitative phosphoproteomics (SILAC)	~7000 phosphorylation sites mapped; shows dynamic MAPK/transcription factor phosphorylation linking to innate immune responses.	20531401
SARS-CoV-2 infection in host cells	Quantitative phosphoproteomics (DIA)	Rewired host phosphorylation networks (p38/MAPK, CK2), altered cell cycle and cytokine production.	32645325
Liver–kidney codysfunction in sepsis	Quantitative phosphoproteomics (DDA)	Metabolic shifts after sepsis in both liver and kidney; disrupted lipid/iron metabolism highlight organ crosstalk.	38581235
Kidney phosphoproteome in sepsis-induced AKI	Quantitative phosphoproteomics (DDA)	Inflammation, pyroptosis, and metabolic alterations identify potential AKI biomarkers and therapeutic targets.	32963032
Interaction proteomics			
NLRP3 inflammasome interactome	Interaction proteomics (AP-MS)	Mapped NLRP3 interactions, revealing novel components in hyperinflammation.	30402268
NFKB1 mutation causing hyperinflammation	Interaction proteomics (AP-MS and proximity labeling)	Identified p.R157X stop-gain mutation causing hyperinflammatory responses and enhanced inflammasome activation	28115215
Identification of truncating *NFKB1* variants causing severe hyperinflammation and necrotizing fasciitis	Interaction proteomics (proximity labeling)	Truncating *NFKB1* variants reduce autophagy, promoting NLRP3/TRIF complex accumulation, elevated IL-1β, and IFN signaling. Patients show hyperinflammation triggered by trauma/infection; IL-1β blockers or JAK inhibitors may mitigate severe necrotizing fasciitis.	38593810
Single-cell proteomics			
SCoPE-MS for single-cell proteomics	Single-cell MS (SCoPE-MS)	Enables quantification of proteins in single cells, analyzing diverse immune responses.	30343672
SCoPE2 for single-cell proteomics	Single-cell MS (SCoPE2)	High-throughput single-cell proteomics reveals immune cell heterogeneity in inflammation.	33504367
Single-cell proteomics in COVID-19 severity	Single-cell proteomics (Olink PEA)	Dysregulation of JAK/STAT, MAPK/mTOR, NF-κB pathways in severe COVID-19, aiding hyperinflammation insight.	35839768
Other COVID-19–related studies			
Large-scale clinical proteomics in COVID-19	Quantitative proteomics (DIA)	27 protein biomarkers differentiate severity; highlight IL-6–centered	32619549

(Continued)

TABLE I. *(Continued)*

Study focus	Proteomics technique	Key findings	Reference (PubMed ID)
		inflammation and complement activation.	
Serum proteome/metabolome in severe vs nonsevere COVID-19	Quantitative proteomics (TMT) and metabolomics	93 proteins and 204 metabolites altered; complement, macrophage/platelet pathways, metabolic suppression noted.	32492406
Multiomic profiling in COVID-19 autopsies	Quantitative proteomics (TMT), transcriptomics, metabolomics, lipidomics	219 molecular features tie to severity; complement activation, lipid transport disruption, neutrophil degranulation, coagulopathy, acute-phase response. Web tool: covidomics.app.	33096026
Multiorgan proteomics autopsy study in COVID-19	Quantitative proteomics (TMT)	11,394 proteins; 5,336 dysregulated. Cathepsin L1 up in lungs instead of ACE2. Systemic hyperinflammation, metabolic and coagulation dysregulation, and fibrosis. Testicular injury noted.	33503446
PBMC proteome in severe COVID-19 vs non–COVID-19 sepsis	Quantitative phosphoproteomics and proteomics (TMT)	Extensive kinase reprogramming (CK2, JAK2/3), abnormal cytokine signaling, dysregulated adaptive/innate immunity.	38389037
Immunopathologic signatures in MIS-C vs pediatric COVID-19	Targeted proteomics (SomaScan PEA) and multiomics	About one third of MIS-C cases show thrombocytopenia and elevated T-cell activation markers (sIL2RA, TNF-α, IFN-γ).	35177862
HLH-like phenotype in a MIS-C subset	Targeted proteomics (Olink PEA)	MIS-C shows hyperinflammation (type II IFN/NF-κB) vs strong type I IFN in pediatric COVID-19.	38744408

ACE2, Angiotensin-converting enzyme 2; *AKI,* acute kidney injury; *ApoC3,* apolipoprotein C-III; *ApoE,* apolipoprotein E; *B2M,* β_2 microglobulin; *CRP,* C-reactive protein; *DDA,* data-dependent acquisition; *mTOR,* mechanistic target of rapamycin; *PCT,* procalcitonin; *SCoPE2,* single-cell proteomics; *SILAC,* stable isotope labeling by amino acids in cell culture; *TMT,* tandem mass tag; *VCAM1,* vascular cell adhesion molecule 1.

variants in 6 unrelated families with severe soft tissue hyperinflammation and necrotizing fasciitis revealed that these variants impair NF-κB1 (p105/p50) function, leading to reduced autophagy. This results in the accumulation of the NLRP3 inflammasome and Toll–IL-1 receptor-domain–containing adaptor-inducing IFN-β (aka TRIF) complexes, elevated IL-1β secretion, and increased type I interferon signaling. Consequently, patients are predisposed to hyperinflammatory episodes triggered by trauma or infection. The results suggest that anti-inflammatory therapies (eg, IL-1β blockers, Janus kinase [JAK] inhibitors) may help mitigate the severe necrotizing fasciitis and systemic cytokine storm seen in these patients.[71]

These findings underscore the importance of protein interactions in maintaining immune homeostasis and illustrate how disruptions in these networks can lead to pathologic inflammation. By providing a detailed map of these interactions, interaction proteomics offers an approach to identify novel therapeutic targets that can modulate these processes. Furthermore, integrating interaction proteomics with other omics approaches, such as genomics, transcriptomics, and/or metabolomics, could enhance our understanding of the multifaceted nature of inflammatory diseases and open new avenues for developing personalized medicine strategies aimed at mitigating these conditions.[68,70]

Phosphoproteomics in hyperinflammation

Phosphoproteomics is a specialized subset of proteomics that focuses on the study of phosphorylated proteins and peptides.[72] The advent of phosphopeptide enrichment techniques along with advancements in high-resolution MS has revolutionized research in this field.[73] This technology is particularly powerful for studying rapid cellular signaling changes occurring during hyperinflammatory conditions, where alterations in protein phosphorylation act as crucial modulators influencing a myriad of signaling pathways that lead to dysregulated immune responses.[74]

Several phosphoproteomic studies have illuminated the central role of phosphorylation in mediating the activity of key proteins such as STAT3 and NF-κB, which are integral to immune and inflammatory signaling.[75,76] Specifically, aberrant phosphorylation of these proteins has been implicated in the pathogenesis of inflammatory diseases like rheumatoid arthritis.[77] Another study applied phosphoproteomics to understand the mechanisms driving acute inflammation. It revealed that the early initiation phase of acute inflammation primarily involved regulating phosphoproteins of glucose metabolism and lipid synthesis pathways. This led to the generation of energy and molecules, such as proinflammatory factors, and the induction of apoptosis. The study demonstrated that metabolism plays a key role in the early stages of acute inflammation, revealing a connection between metabolic pathways and hyperinflammation.[78]

Further highlighting the value of phosphoproteomics is a study that used stable isotope labeling by amino acids in cell culture–based analyses of LPS-stimulated macrophages to map ~7000 phosphorylation sites, linking dynamic mitogen-activated protein kinase (MAPK)/transcription factor phosphorylation to innate immune responses.[79] In SARS-CoV-2 infection, DIA phosphoproteomics revealed rewired host phosphorylation networks

(p38/MAPK, protein kinase 2 [CK2]) altering cytokine production.[78] Phosphoproteomics has also exposed metabolic shifts in sepsis-induced liver–kidney codysfunction[80] and identified potential acute kidney injury biomarkers in sepsis-induced acute kidney injury through inflammation and pyroptosis-related changes.[81]

By shedding light on these nuanced molecular mechanisms, phosphoproteomics has significantly contributed to our understanding of hyperinflammation and is guiding the development of innovative strategies for diagnosis and treatment.

Affinity-based proteomics in hyperinflammation

Over the last few years, scientific advancements outside of the MS realm have also enabled the development of high-throughput targeted proteomic assays using protein-specific affinity reagents.[82] Currently, the two prominent methodologies, PEA and aptamer-based protein assays, both rely on specific affinity reagents to quantify large panels of circulating proteins from blood with minimal sample volumes. Although they differ in detection chemistry (PEA uses paired antibodies with complementary oligonucleotides, while aptamer-based assays use modified DNA ligands), both methods enable the simultaneous measurement of numerous cytokines, chemokines, and other proteins. Recently, the majority of affinity-based proteomics studies on hyperinflammation have focused on multisystem inflammatory syndrome in children (MIS-C), as exemplified by two comprehensive investigations that used both Olink PEA and Somalogic aptamer platforms.[83,84] While these and similar studies underscore the heightened cytokine response typical of MIS-C, they also occasionally report differing sets of elevated biomarkers, even within the same platform. Such variations may be attributed to differences in the design and timing of the assay panels, the rapidly evolving analytic pipelines, and cohort-level heterogeneity (eg, age, genetics, disease phase). Despite these discrepancies, affinity-based proteomic assays will likely develop to be indispensable for elucidating inflammatory pathophysiology from human blood.

Single-cell proteomics in hyperinflammation

Single-cell proteomics is an emerging field that advances the study of proteomes to the individual cell level.[85,86] Several techniques have been developed, including MS-based methods such as single-cell proteomics by MS,[87] and antibody-based approaches like cytometry by time of flight (aka CyTOF).[88,89] The ability to dissect proteomic profiles of individual cells provides a granular view of the cellular landscape, shedding light on the functional diversity and specialization within immune cell populations.[90,91] For instance, single-cell proteomic analysis was used to identify and characterize subsets of macrophages that produce specific inflammatory mediators.[91] This enhanced our understanding of cellular heterogeneity and functional specialization in inflammation. These insights hold promise for identifying novel therapeutic targets and developing precision medicine strategies.[92]

In the context of COVID-19, a study applied single-cell proteomics to analyze over 1,400 plasma proteins and 2,600 single-cell immune features in peripheral blood from 97 patients with varying disease severity. The study identified distinct biological signatures correlating with disease severity. Notably, severe COVID-19 cases were associated with marked dysregulation of key immune signaling networks including JAK/STAT, MAPK/mammalian target of rapamycin (aka mTOR), and NF-κB pathways. Additionally, increasing disease severity correlated with the enrichment of certain plasma proteins and diminished intracellular signaling responses in immune cells. These findings provided deeper insights into the pathophysiology of COVID-19 and identified potential biomarkers and therapeutic targets for predicting and managing disease progression.[93]

Similarly, to investigate inflammatory mechanisms in ulcerative colitis, researchers utilized high-dimensional single-cell proteomics analyses. By generating a dataset from peripheral blood mononuclear cells (PBMCs) of healthy individuals and ulcerative colitis patients, and using mass cytometry, they performed system-wide analyses of immune cell frequencies and cell type–specific expression patterns of 12 immune checkpoints. This study revealed significant changes in immune cell frequencies in ulcerative colitis patients, most notably reduced levels of peripheral NK cells that expressed an increase in TIGIT, which regulates NK cell activity, thereby presenting a potential therapeutic target for development.[94]

Role of proteomics in disease diagnostics and prognostics

Proteomics, which encompasses the comprehensive profiling of protein expression, temporal changes, and posttranslational modifications, plays a key role in prognosticating diseases, particularly the large and variable group of diseases with inflammatory and hyperinflammatory states.[95,96] The ability to quantify and monitor protein dynamics provides critical insights into disease progression and patient outcomes.

A prominent example is the application of proteomic studies in patients with severe COVID-19.[66,97-100] These studies uncovered specific protein signatures, such as the elevation of acute-phase proteins and dysregulated cytokines, that correlate with disease severity and clinical outcomes. Proteomic data have been used to develop prognostic models that serve as invaluable guides for clinicians, enabling the anticipation of patient trajectories and the strategic formulation of treatment plans.[66] Moreover, real-time feedback from proteomic analyses is instrumental in assessing the efficacy of therapeutic interventions, facilitating timely adjustments to optimize patient care.[66] The integration of proteomics in prognostics exemplifies a convergence of precision and adaptability, marking a significant stride in personalized medicine and offering hope for patients navigating the complexities of hyperinflammatory conditions.

In chronic inflammatory diseases, proteomics has been key in identifying novel biomarkers for diagnosis and prognosis. The analysis of a wide array of proteins in tissues and body fluids has bridged the gap between genomic/transcriptomic data and the phenotypic presentation of complex diseases with inflammatory and hyperinflammatory components, such as multiple sclerosis, rheumatic diseases, and pulmonary inflammatory diseases. For instance, proteins like the heat shock protein family in multiple sclerosis and myeloid-related protein 8 in rheumatoid arthritis have emerged as potential biomarkers, aiding in disease monitoring and therapeutic decision-making.[101]

Furthermore, targeted serum proteomics has been used to differentiate patients with various subtypes of CVID, highlighting the accuracy and reproducibility of this technique in dissecting

inflammatory pathways.[64] In ischemic heart disease, the interplay between inflammation and microvascular dysfunction has been a focus of proteomic research, underlining its potential in understanding disease progression and management.[102] In the context of inflammatory bowel disease, proteomics has provided a deeper understanding of disease mechanisms and aided in the identification of new biomarkers crucial for diagnosis and management.[95,103] Similarly, in chronic kidney disease, advancements in omics, including proteomics, have been instrumental in elucidating disease pathophysiology. The identification of biomarker panels through proteomic analyses has enhanced early diagnosis, monitoring, and prognostication.[104]

In COVID-19, large-scale DIA proteomics identified 27 protein biomarkers differentiating disease severity, centering on IL-6 and complement activation.[96] Proteome/metabolome combined analysis in severe vs nonsevere COVID-19 (quantitative proteomics and metabolomics) showed 93 proteins and 204 metabolites altered, noting complement, macrophage/platelet pathways, and metabolic suppression.[105] Multiomic profiling of samples collected during COVID-19 autopsies via quantitative proteomics and multiomics linked complement activation and lipid disruption to disease severity.[106] Another multiorgan proteomics autopsy study in COVID-19 identified 11,394 proteins with 5,336 dysregulated, highlighting cathepsin L1 over angiotensin-converting enzyme 2 in lung and systemic hyperinflammation.[107] Finally, a PBMC proteome comparison in severe COVID-19 versus non–COVID-19 sepsis revealed extensive kinase reprogramming (eg, CK2, JAK2/3) and dysregulated adaptive/innate immunity.[108] Collectively, these examples demonstrate the practical applications of proteomics approaches in the prognosis of chronic, inflammatory, and hyperinflammatory diseases.

Challenges and future directions in proteomics of hyperinflammation

As biomedical research advances, proteomics, including quantitative proteomics, phosphoproteomics, interaction proteomics, and single-cell proteomics, is expanding our understanding of inflammation and hyperinflammation. Almost two decades ago, proteomics and systems biology were proposed to address key biomedical questions such as elucidating the causes of sepsis.[109] Since then, significant advancements have been made, with proteomics providing detailed insights into protein dysregulation and the complex cellular heterogeneity inherent in a broad range of inflammatory and hyperinflammatory disorders.

Specifically, quantitative proteomics has revealed changes in protein abundance and posttranslational modifications critical for understanding inflammatory responses. Phosphoproteomics has elucidated cellular signaling alterations, highlighting mechanisms regulating inflammation. Interaction proteomics has mapped complex protein–protein interaction networks, uncovering how disruptions contribute to pathologic inflammation. And single-cell proteomics has offered unparalleled resolution of the cellular landscape, exposing the functional diversity and specialization of immune cells and presenting a detailed orchestration of the immune response.

The combined application of these proteomic techniques holds promise for significant scientific and clinical advancements. They will facilitate the identification of novel therapeutic targets and foster personalized treatment strategies, aiming to improve the prognosis and quality of life for patients with hyperinflammatory diseases. However, integrating proteomic insights into clinical practice presents several challenges.

Technical hurdles. The need for standardization of methodologies, enhancement of sensitivity and specificity in protein detection, and reproducibility across laboratories is paramount. The development of sophisticated computational and bioinformatics tools is essential for interpreting the complex and high-dimensional data generated by proteomic studies. Integrating proteomic data with other omics datasets (genomics, transcriptomics, metabolomics) requires advanced analytic frameworks and interdisciplinary collaboration.

Ethical concerns. Concerns around data privacy and security in particular must be addressed to ensure the responsible use of proteomic data. The handling of patient-derived proteomic information necessitates stringent protocols to protect confidentiality and comply with regulatory standards.

Overcoming these obstacles and leveraging the strengths of proteomic technologies could usher in an era where precision medicine in treating hyperinflammatory conditions becomes reality. As proteomics leads the way in the broader context of multiomics research, it promises to provide in-depth and integrated understanding of disease processes, marking a transformative shift in the therapeutic approach to inflammatory and hyperinflammatory diseases.[110]

METABOLOMICS AND HYPERINFLAMMATION

Metabolomics is a vital branch of omics technologies that involves the comprehensive study of metabolites, or small molecules produced through metabolic processes within an organism.[111,112] These metabolites are end products of cellular activities and provide a direct reflection of the physiologic state of a cell, tissue, or organism. Metabolomics enables the exploration of how organisms respond to internal (genetic) and external (environmental) stimuli, offering invaluable insights into the metabolic alterations underpinning hyperinflammation. We turn next to an exploration of the application of metabolomics in investigating metabolic dysregulation in inflammation and hyperinflammation, and its emerging role in disease diagnostics (Table II).

Metabolic dysregulation in hyperinflammatory disorders

Hyperinflammatory conditions are marked by profound disruptions in metabolic pathways. These metabolic changes are not only consequences of the inflammatory process but also contribute to its progression. Metabolomics offers a powerful framework to assess these alterations, providing an extensive biochemical snapshot of the hyperinflammatory state.

Many studies have highlighted the crucial interplay between metabolic regulation and cytokine release, which is the hallmark of hyperinflammation.[113-115] For instance, the key proinflammatory cytokine IL-6 modulates glucose metabolism and regulates immunometabolic reprogramming during acute stress conditions.[116] Metabolomics has also facilitated the identification of specific metabolic pathways critical in modulating host responses to viral infections,[117] including those caused by influenza viruses[118] and SARS-CoV-2.[119] For example, untargeted

TABLE II. Metabolomics studies providing insights into hyperinflammation and inflammation

Study focus	Metabolomics technique	Key findings	Reference (PubMed ID)
Untargeted metabolomics			
Accelerated untargeted metabolite identification	Untargeted (MS/MS)	Rapid automated metabolite identification reduces analysis from days to hours; improved coverage in serum and *Escherichia coli* extracts.	22965049
Itaconate's anti-inflammatory role in macrophages	Untargeted (MS/MS), isotope tracing	Itaconate (Irg1 derived) suppresses LPS-induced inflammation via Nrf2 activation; reduces IL-1β, ROS, and lethality *in vivo*.	29590092
Predicting TB progression from metabolic changes	Untargeted (MS/MS, GC-MS)	Cortisol, kynurenine, and amino acid shifts predict latent TB progression months before clinical signs; metabolic biosignature improves risk stratification.	30523338
Malaria-infected children's metabolic response	Untargeted (MS/MS), transcriptomics)	Sepsis-like metabolic changes; depletion of acylcarnitines, increased steroids. Steroid-driven T-cell suppression and ethnic differences influence susceptibility.	34113019
Distinct immune states in sepsis (MALS, etc)	Untargeted (MS/MS)	MALS (hyperinflammation) exhibits largest metabolic disruption; other immune states show fewer alterations.	39418210
Spns2/S1P signaling and PGE2 regulation	Untargeted (MS/MS)	Spns2 deficiency elevates PGE2, impairing mitochondrial respiration, intensifying early inflammation and later immunosuppression.	39350143
COVID-19 severity biomarkers (untargeted and lipids)	Untargeted (high-resolution MS metabolomics and lipidomics)	Kynurenine pathway, amino acid/fatty acid disruption in severe COVID-19; identified biomarker panel enhances severity prediction.	39636373
GPP metabolome and IL-1β regulation (combo)	Untargeted (GC-MS) and targeted (MS/MS)	GPP exhibits broad amino acid depletion, activating GCN2/AAR in monocytes, reducing IL-1β and controlling hyperinflammation. (Also in Targeted)	34567002
Severe COVID-19 immunometabolism (combo)	Combined targeted/untargeted (MS/MS)	Severe COVID-19 shows global metabolic disruptions (arginine, tryptophan, purines) linked to cytokine storms; metabolic interventions reduce cytokine release. (Also in Targeted)	33712622
Targeted metabolomics			
Itaconate and derivatives in immunoregulation	Targeted metabolomics (MS/MS)	Itaconate/dimethyl-itaconate conjugate glutathione, inducing electrophilic stress and Nrf2 response, selectively inhibiting IL-6 and secondary inflammatory genes.	29670287
GPP metabolome and IL-1β regulation (combo)	Targeted metabolomics (MS/MS) and untargeted (GC-MS)	GPP's amino acid depletion reduces IL-1β output via GCN2/AAR activation, controlling hyperinflammation. (Also in Untargeted)	34567002
Severe COVID-19 immunometabolism (combo)	Combined targeted/untargeted (MS/MS)	Severe COVID-19: metabolic disruption correlates with cytokine storm; targeted interventions reduce cytokine release. (Also in Untargeted)	33712622
Comparing ARDS etiologies (COVID-19, H1N1, etc)	Targeted metabolomics (MS/MS)	Distinct metabolic phenotypes differentiate ARDS causes: COVID-19 vs H1N1 differ in taurine, TCA intermediates; COVID-19 vs bacterial ARDS differ in amino acid metabolism; severity markers for COVID-19 ARDS.	31142855
Fluxomics			
Succinate as inflammatory signal in LPS macrophages	LC-MS metabolite profiling, flux analysis	LPS induces glycolysis and succinate accumulation, stabilizing HIF-1α and boosting IL-1β. Blocking succinate reduces IL-1β.	23535595

ARDS, Acute respiratory distress syndrome; *Combo,* combination study; *GC-MS,* gas chromatography–MS; *GCN2/AAR,* general control nonderepressive 2/amino acid stress response; *GPP,* generalized pustular psoriasis; *HIF-1α,* hypoxia-inducible factor 1-alpha; *Irg1,* immune responsive gene 1; *LC-MS,* liquid chromatography–tandem MS; *MALS,* macrophage activation-like syndrome; *mTOR,* mechanistic target of rapamycin; *Nrf2,* nuclear factor–like 2; *PGE2,* prostaglandin E_2; *ROS,* reactive oxygen species; *S1P,* sphingosine-1-phosphate ; *SCoPE-MS,* single-cell proteomics by MS; *Spns2,* spinster homolog 2; *TB,* tuberculosis; *TCA,* tricarbolic acid; *TRIF,* Toll–IL-1 receptor-domain–containing adaptor-inducing IFN-β.

metabolomics approaches have revealed that changes in cortisol, kynurenine, and amino acids can predict the transition from latent to active tuberculosis months before the manifestation of clinical symptoms.[120] Another study utilized untargeted metabolomics to demonstrate infection-driven metabolic alterations during malaria, identifying a role for steroid-related immunosuppression.[121] These findings emphasize that metabolic dysregulation is a central feature of hyperinflammatory states and can precede overt clinical symptoms.

Metabolomics research can be broadly categorized into 3 main approaches: untargeted, targeted, and fluxomics. First, untargeted metabolomics captures a wide spectrum of metabolites without prior knowledge, enabling the discovery of novel metabolic changes and unexpected biomarkers in hyperinflammatory conditions. For instance, untargeted approaches identified itaconate as a crucial anti-inflammatory metabolite derived from Irg1 that suppresses LPS-induced inflammation.[115] Second, targeted metabolomics focuses on quantifying specific metabolites. By validating findings from untargeted studies, targeted techniques have elucidated pathways like the general control nonderepressive 2/amino acid stress response in generalized pustular psoriasis, where amino acid depletion diminishes IL-1β secretion and hyperinflammation.[122] Third and last, fluxomics investigates the rates of metabolic reactions in real time. This approach revealed that LPS induces glycolysis and succinate accumulation in macrophages, stabilizing hypoxia-inducible factor 1-alpha (aka HIF-1α) and enhancing IL-1β production.[113] Such real-time insights are critical for understanding the dynamic nature of hyperinflammatory responses.

Emergence of metabolomics in disease diagnostics

The application of metabolomics in disease diagnostics has garnered significant attention, particularly for hyperinflammatory disorders. Metabolomics enables the identification of unique metabolic fingerprints that serve as potential biomarkers for diagnosis and prognosis. These biomarkers reflect real-time physiologic and pathologic processes, making them exceptionally valuable for early detection, monitoring, and therapeutic guidance.

In COVID-19, a condition characterized by hyperinflammation, metabolomics studies have revealed global metabolic disruption, including alterations in arginine, tryptophan, and purine metabolism, which correlate with cytokine storms.[123] Another untargeted metabolomics and lipidomics study in COVID-19 identified disruptions in amino acid and fatty acid metabolism and sphingolipid alterations, ultimately leading to a biomarker panel that refines severity prediction.[124] These examples illustrate how metabolomics offers a molecular window into the underlying metabolic chaos driving hyperinflammation.

Similarly, metabolomics identified metabolic shifts in *Mycobacterium tuberculosis* infection well before clinical symptoms arise, including changes in cortisol, kynurenine, and amino acids.[120] Such early metabolic alterations can potentially guide clinical decision-making, thereby improving patient outcomes. Furthermore, global metabolomics has been utilized to identify steroids as key metabolites influencing the immune response during infection by the malaria-causing parasite *Plasmodium falciparum*.[121,125]

Metabolomics and clinical management of hyperinflammatory disorders

Beyond diagnosis, metabolomic signatures offer significant potential for monitoring disease progression and therapeutic response. For instance, characterizing distinct immune states in sepsis through metabolic profiles demonstrated that macrophage activation-like syndrome exhibits profound metabolic changes.[126] Understanding these changes can guide personalized therapies by adjusting interventions on the basis of a patient's unique metabolic profile.

However, clinical application faces challenges, including the dynamic nature of the metabolome, sample processing complexities, and the need for large-scale validation.[127] Nonetheless, advancements in analytic techniques and computational tools have expanded the scope of metabolomics.[120,128-130] Further, integrating metabolomics with other omics—genomics, transcriptomics, and proteomics—has fueled comprehensive insights into hyperinflammatory disorders, revealing mechanistic links and identifying novel therapeutic targets.[131-133]

As technology and data analytics evolve, metabolomics could significantly transform hyperinflammatory disorder management, enabling earlier diagnosis, tailored therapies, and improved prognostication, ultimately improving patient care.

MULTIOMICS, SYSTEMS BIOLOGY, AND ARTIFICIAL INTELLIGENCE IN HYPERINFLAMMATION

Hyperinflammation is particularly evident in the context of cytokine storms, where the immune system's response becomes excessive and uncontrolled. The intricacies of cytokine storms highlight the complexity of immune responses and their regulation, pointing toward a fundamental systems-level gap in a complete understanding of these processes.[134-137] Addressing this gap requires synergy between computational and experimental research. Advances in technology have enabled a new era where computational models and experimental data converge to facilitate a deeper understanding of, and more precise interventions in, hyperinflammatory conditions. This intersection of computational biology with traditional experimental methods opens doors to new discoveries and therapeutic strategies.[138-140]

In the realm of systems biology, the generation of extensive omics datasets has significantly contributed to our understanding of hyperinflammation. These large-scale datasets have been used to develop sophisticated computational models, paving the way for more effective and targeted therapies to combat hyperinflammation.[141-143] For example, in a recent computational study, the inference and analysis of functional cell–cell communication networks allowed the reconstruction of the positive feedback loops involved in maintaining and amplifying the hyperinflammatory immune response. This led to the identification of potential targets for modulating the inflammatory response in critically ill patients with COVID-19.[144] Furthermore, a single-cell transcriptomics approach identified a low proportion of MerTK-positive synovial tissue macrophages associated with an increased risk of rheumatoid arthritis flare after treatment cessation, constituting a target subpopulation for potential therapeutic strategies.[145] More recently, a systems immunology-based

drug repurposing approach was developed to reduce inflammation in atherosclerotic cardiovascular disease.[146] However, challenges persist in building computational models for hyperinflammation. Issues such as accessing tissue-specific or location-specific data, effectively integrating time-series data, and determining the optimal timing for intervention strategies represent significant hurdles. Addressing these challenges is essential for advancing our understanding and treatment of hyperinflammatory conditions.

With recent advancements in artificial intelligence (AI) technologies, machine learning approaches that integrate and analyze various omics data are becoming increasingly valuable for drug repurposing in the context of inflammatory diseases (Fig 2). One study on rheumatoid arthritis used machine learning models built using a random forest algorithm on gene expression and DNA methylation profiles from patients' PBMCs, monocytes, and $CD4^+$ T cells to predict response to anti-TNF treatment, which was further validated in a follow-up study.[147] In another study, a deep learning methodology that integrates networks between drug, gene, and disease datasets was implemented for drug repurposing. This approach identified a potential therapeutic effect for topotecan, an approved topoisomerase inhibitor and direct inhibitor of human retinoic acid receptor–related orphan receptor gamma t (aka ROR-γt), in a mouse model of multiple sclerosis.[148] Furthermore, a computational platform for drug repurposing, which integrates transcriptomic, proteomic, and structural data within a principled causal framework, was applied in the context of SARS-CoV-2. By using an autoencoder—a type of artificial neural network used to learn efficient data representations in an unsupervised manner—the authors were able to embed the Connectivity Map data[149] together with SARS-CoV-2 expression data for signature matching. This resulted in an ordered list of drugs approved by the US Food and Drug Administration that could be used to treat COVID-19 patients.[150]

In proteomics, the accurate measurement of protein expression and proteoforms will provide vast opportunities for the development of AI-based integration models. Proteomics data can serve as a readout after experimental procedures that enrich for specific functional subclasses of proteins. For instance, interactomics involves pulling down interacting proteins with a bait, such as a protein of interest or a small-molecule drug, leading to a network of interactions with perturbations that can be studied using AI and network analysis methods.[151] The cellular proteome can also be spatially separated by centrifugation followed by MS-based proteomics to map the spatial protein compositions of specific organelles. Moreover, MS-based proteomics has revealed the complex regulation of proteins by posttranslational modifications, highlighting the need for using AI to integrate proteomics data with other omics data or clinical information.[152] Although AI applications in proteomics are in their infancy, their integration with genomics, metabolomics, lipidomics, and clinical data holds promise for advancing our understanding of hyperinflammatory conditions.[153,154]

Despite the increasing use of machine learning methods for drug repurposing to reduce hyperinflammation, these approaches require vast amounts of data to establish statistical relationships between input data and predicted outputs. Additionally, these methods are often considered "black boxes," focusing on predictions without explicitly explaining the rationale behind the results. To address these issues, new developments in interpretable and causal learning algorithms are being pursued. Furthermore, combining machine learning approaches with mechanistic models based on gene regulation and signaling networks can provide more insights into drug mechanisms of action, including information on targeted signaling pathways and downstream effects on gene regulation.

FROM RARE TO COMMON: A RECAP

Hyperinflammation, once perceived as a rare and isolated phenomenon, has now emerged as a central feature in a wide array of diseases, including autoimmune disorders and severe infections like COVID-19. This shift in understanding is largely attributed to advancements in omics technologies, which have provided profound insights into the genetic, proteomic, and metabolic underpinnings of hyperinflammatory responses. These technologies have revealed the intricate molecular mechanisms that drive these conditions and have the potential to transform their diagnosis and treatment.

Genomic studies have identified critical mutations and polymorphisms that predispose individuals to severe inflammation and hyperinflammation. Proteomic analyses have unveiled specific protein signatures and phosphorylation events that characterize these states, while metabolomics has highlighted the metabolic disruptions occurring during hyperinflammatory episodes. Together, these omics approaches have not only broadened our understanding of inflammation and hyperinflammation but also paved the way for novel diagnostic and therapeutic strategies.

PROMISING HORIZON OF OMICS IN HYPERINFLAMMATION RESEARCH AND TREATMENT

The integration of omics technologies into clinical practice holds immense potential for transforming the management of hyperinflammatory diseases. A first glimpse of this has already been seen with COVID-19. By enabling precise diagnostics and personalized treatment plans, these approaches can significantly improve patient outcomes. For example, stratifying patients according to their molecular profiles could allow for more targeted and effective interventions, reducing the risk of adverse effects and enhancing therapeutic efficacy.

Moreover, the synergy between computational and experimental research is driving the development of sophisticated models that can better predict disease progression and treatment responses. The application of AI, particularly machine learning, in analyzing multiomics datasets is opening new avenues for drug discovery and repurposing, further expanding the therapeutic arsenal against hyperinflammation.

FUTURE DIRECTIONS AND CHALLENGES

Despite significant advancements, several challenges remain in hyperinflammation research. Technical hurdles, such as the need for standardization of omics methodologies and the development of robust bioinformatics tools, must be addressed to fully realize the potential of these technologies. Additionally, ethical considerations related to data privacy and the responsible use of genetic information are paramount and require careful navigation. This is particularly important given the increased use of long-read

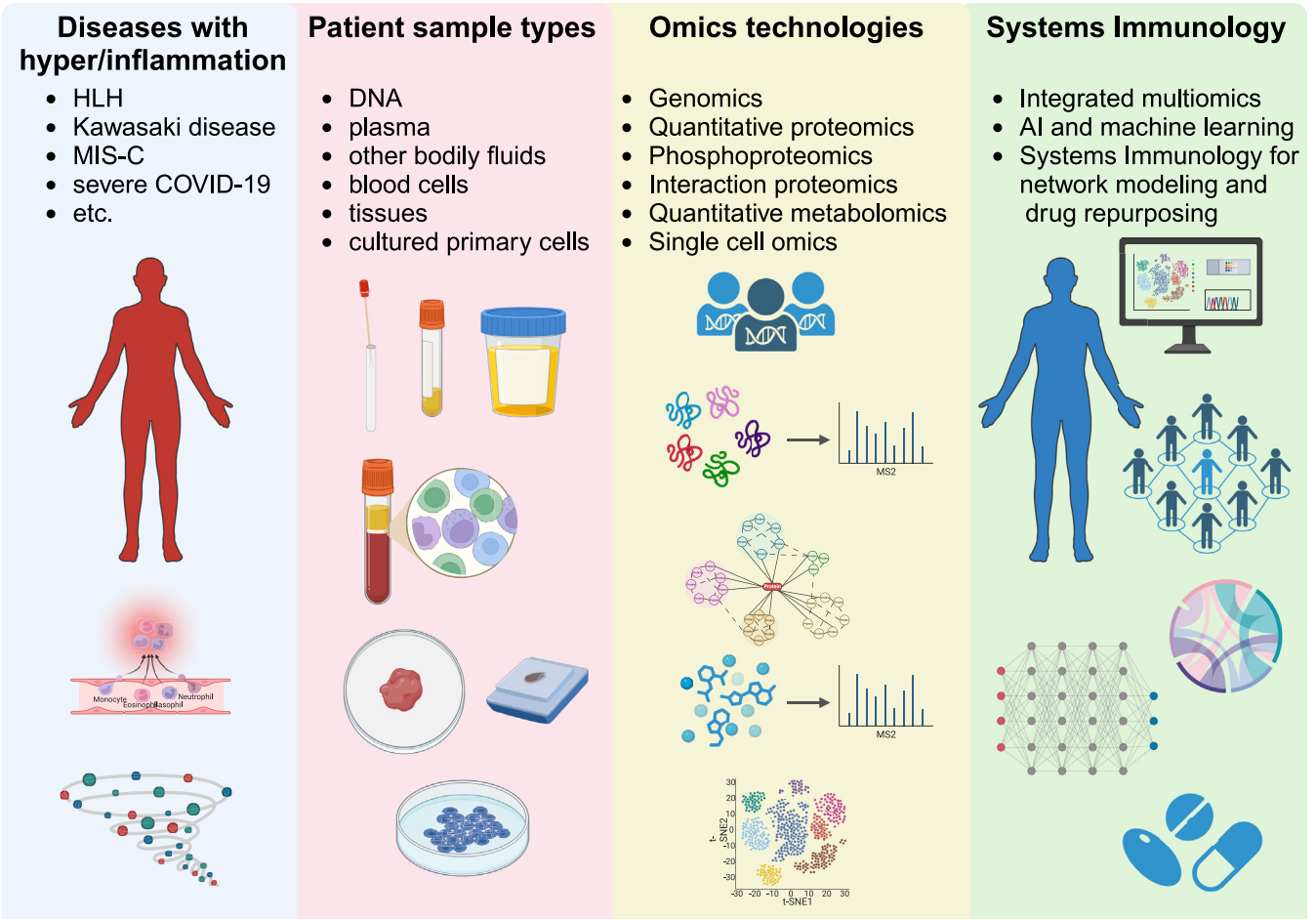

FIG 2. Application of omics technologies and systems immunology in hyperinflammation research. Various biospecimens (DNA, plasma, body fluids, blood cells, tissues) can be probed using cutting-edge omics platforms: genomics, quantitative proteomics, phosphoproteomics, interaction proteomics, metabolomics, and single-cell omics. Together, these methods offer integrated, high-resolution portrait of molecular dysregulation underlying hyperinflammatory states. By combining multiomics data with AI, machine learning, and systems immunology approaches, researchers can discover new biomarkers, build mechanistic disease models, and accelerate identification of novel or repurposed therapies. This framework has been widely applied to hyperinflammatory conditions such as HLH, Kawasaki disease, MIS-C, and severe COVID-19.

whole-genome sequencing to unravel deep intronic genetic mechanisms.[3]

Future research should focus on overcoming these challenges and expanding the application of omics technologies to a broader range of inflammatory conditions. Collaborative efforts among researchers, clinicians, and policymakers will be crucial in translating these scientific discoveries into tangible clinical benefits.

In conclusion, the journey from understanding hyperinflammation as a rare phenomenon to recognizing it as a feature in many diseases has been transformative. The continued advancement and integration of omics technologies holds the promise of ushering in a new era of precision medicine, where hyperinflammatory conditions are managed with unprecedented accuracy and efficacy. As we stand on the cusp of this new frontier, the insights gained from omics research offer hope for patients and a roadmap for future scientific exploration.

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