Cytokine and Growth Factor Reviews 77 (2024) 1–14

Contents lists available at ScienceDirect

Cytokine and Growth Factor Reviews

journal homepage: www.elsevier.com/locate/cytogfr

Unveiling cytokine charge disparity as a potential mechanism for immune regulation

Jennifer M. Messina [a], Minghao Luo [b], Md Shanewaz Hossan [a], Hadil A. Gadelrab [a], Xiguang Yang [a], Anna John [a], Joel R. Wilmore [c,f], Juntao Luo [a,c,d,e,f,*]

[a] Department of Pharmacology, State University of New York Upstate Medical University, Syracuse, NY 13210, United States
[b] Department of Clinical Medicine, 2nd Affiliated Hospital, Harbin Medical University, Harbin, Heilongjiang 150081, China
[c] Department of Microbiology and Immunology, State University of New York Upstate Medical University, Syracuse, NY 13210, United States
[d] Department of Surgery, State University of New York Upstate Medical University, Syracuse, NY 13210, United States
[e] Upstate Cancer Center, State University of New York Upstate Medical University, Syracuse, NY 13210, United States
[f] Upstate Sepsis Interdisciplinary Research Center, State University of New York Upstate Medical University, Syracuse, NY 13210, United States

ARTICLE INFO

Keywords:
Cytokine
Isoelectric point
Charge disparity
Tissue residence
Pharmacokinetics

ABSTRACT

Cytokines are small signaling proteins that regulate the immune responses to infection and tissue damage. Surface charges of cytokines determine their in vivo fate in immune regulation, e.g., half-life and distribution. The overall negative charges in the extracellular microenvironment and the acidosis during inflammation and infection may differentially impact cytokines with different surface charges for fine-tuned immune regulation via controlling tissue residential properties. However, the trend and role of cytokine surface charges has yet to be elucidated in the literature. Interestingly, we have observed that most pro-inflammatory cytokines have a negative charge, while most anti-inflammatory cytokines and chemokines have a positive charge. In this review, we extensively examined the surface charges of all cytokines and chemokines, summarized the pharmacokinetics and tissue adhesion of major cytokines, and analyzed the link of surface charge with cytokine biodistribution, activation, and function in immune regulation. Additionally, we identified that the general trend of charge disparity between pro- and anti-inflammatory cytokines represents a unique opportunity to develop precise immune modulation approaches, which can be applied to many inflammation-associated diseases including solid tumors, chronic wounds, infection, and sepsis.

1. Introduction

Inflammation usually serves a protective role in minimizing injury or infection. Pathogen-Associated Molecular Patterns (PAMPs) from microbial structures or endogenous Damage-Associated Molecular Patterns (DAMPs) released in tissue damage initiate the inflammatory response through interaction with Pattern Recognition Receptors (PRRs) [1] on immune cell surfaces to activate inflammatory pathways. Both innate and adaptive immune cells are orchestrated to initiate, regulate, and resolve inflammatory responses via secreting and responding to a profile of inflammatory signaling molecules, i.e. cytokines, to activate and recruit more immune cells to the site of inflammation [2]. Cytokines bind to cytokine receptors on immune cells in an autocrine or paracrine manner to further manipulate the inflammatory response. Pro-inflammatory cytokines induce further inflammatory responses, while anti-inflammatory cytokines act as regulatory signals to restore immune homeostasis.

Activation of immune cells induces a shift from oxidative phosphorylation towards aerobic glycolysis with an increase in lactate production [3], like the Warburg effect observed in tumor cells [3,4]. The local increases in lactic acid and protons result in an acidic microenvironment [5]. Acidotic extracellular pH levels, which can range from pH 5.5–7.0, are commonly found in inflammatory conditions such as tumors [6], autoimmune diseases [7], and sites of infection [8]. It has been well

Abbreviations: pI, isoelectric point; IL, interleukin; PAMPs, Pathogen-Associated Molecular Patterns; DAMPs, Damage-Associated Molecular Patterns; PRRs, Pattern Recognition Receptors; ROS, reactive oxygen species; NET, neutrophil extracellular trap; ECM, extracellular matrices; PTM, post-translational modifications; GAGs, glycosaminoglycans; PK, pharmacokinetics; $t_{1/2}$, half-life; MMPs, matrix metalloproteases; GPCRs, G protein coupled receptors.

* Corresponding author at: Department of Pharmacology, State University of New York Upstate Medical University, Syracuse, NY 13210, United States.
E-mail address: luoj@upstate.edu (J. Luo).

https://doi.org/10.1016/j.cytogfr.2023.12.002
Received 20 November 2023; Received in revised form 21 December 2023; Accepted 22 December 2023
Available online 26 December 2023
1359-6101/© 2023 Elsevier Ltd. All rights reserved.

documented that acidosis results in complex changes of immune cell activity [9]. In neutrophils, extracellular acidosis has been shown to increase cell activation, trans-differentiation, endocytosis/phagocytosis, and antigen presenting capacity, while decreasing neutrophil extracellular trap (NET) formation, apoptosis, reactive oxygen species (ROS) production, and cell migration [10–15]. In the case of monocytes and macrophages, acidosis has a complex effect on cellular activity. On one hand, decreased extracellular pH increases inflammasome activation and IL-1β production [16,17], however, it also reduces monocyte recruitment and production of inflammatory mediators such as TNFα and IL-6 [18] and reduces responsiveness of macrophages to LPS stimulation [19,20]. Similarly, acidosis has both pro- [21,22] and anti-inflammatory effects [23,24] on dendritic cells. In the case of natural killer cells, low pH acts in an anti-inflammatory manner through inhibition of critical functions, including release of perforin and granzyme granules, secretion of pro-inflammatory cytokines, and cytotoxic response including anti-tumor immunity[25,26]. In summary, inflammatory acidosis and lactate have pleiotropic effects on immune cells in inflammation.

Cytokines are classified as interleukins, colony stimulating factors, interferons, tumor necrosis factors, tumor growth factors, and chemokines [27]. Cytokines are extracellular signaling proteins, which can be affected by environmental changes such as alterations in pH. Specifically, pH changes modify the charge distribution of a protein, and thus may alter protein geometry and interfere with the electrostatic interactions in protein binding [28,29]. The slight decrease in pH from 6.7 to 6.0 significantly reduces the solubility of negatively charged protein, e.g. insulin [30]. In particular, imidazole in histidine residues has a pKa around 6.0, which can be very sensitive to inflammatory acidosis in local tissue (pH 5.5–7.0) and even systemic pH changes in sepsis (pH 7.0–7.2) [31]. The surface charges of cytokines, indicated by the isoelectric point (pI), stabilize proteins in aqueous solution and can contribute to the receptor binding. Thus, inflammatory acidosis has the potential to modify cytokines in terms of their stability, biodistribution, and affinity for receptor binding. Surprisingly, this phenomenon has not been discussed in the literature. In addition, abundant serum proteins and plasma membranes are mostly negatively charged, and the extracellular matrix and cell surface are intrinsically decorated with negatively charged polysaccharides, e.g., heparin and hyaluronic acids. This negatively charged physiological matrix constantly influences the trafficking and biodistribution of cytokines as they make their way to their targets. **Hence, cytokines may acquire distinct surface charges as part of their evolutionary adaptation, allowing them to efficiently carry out and regulate their functions through interacting with extracellular matrices (ECMs) and responding to immunological and pathological acidosis.**

Interestingly, we have observed a significant charge disparity between major pro-inflammatory and anti-inflammatory cytokines, which provides us an opportunity for precise immune modulation in sepsis treatment [32]. Major pro-inflammatory cytokines, e.g., TNF-α, IL-1β and IL-6, have negative charges. This may be important for pro-inflammatory cytokines to avoid nonspecific interactions with extracellular molecules/matrix allowing for the effective initiation of an immune response against infection and limit tissue damage. In contrast, anti-inflammatory cytokines, e.g., IL-10, IL-4, and TGF-β, are predominantly positively charged resulting in longer residence via charge interactions with negatively charged ECMs for prolonged and effective anti-inflammatory effects. Overwhelming inflammation induced by sepsis or other inflammatory diseases can have detrimental effects such as multiple organ failure. Thus, multiple mechanisms for effective immune regulation are essential to control the side effects of inflammation. Additionally, inflammatory acidosis may serve as an intrinsic regulatory mechanism to prevent overwhelming inflammation through suppressing immune cell activity and decreasing the activity of a broad spectrum of the negatively charged proinflammatory cytokines. However, prolonged acidosis may increase anti-inflammatory cytokine activity and residency to result in immune suppression in the later stage of sepsis and contribute to the immune suppressive microenvironment in solid tumors.

It is difficult to separate the activity of cytokines from the cell responses that occur from contact with environments undergoing immunological and pathological pH changes. In addition, many cytokines share overlapping and redundant signals during inflammation [33]. Bioinformatic analysis of intracellular proteomes revealed an interesting correlation between protein PI and subcellular localization, which is defined by the local pH and membrane charge [34]. Thus, we would like to investigate whether cytokines with overlapping and similar functions also share a similar surface charge, which allows for a universal pathway for immune regulation in response to acidosis. Although there are no specific studies exploring the potential significance of cytokine charge disparities, it is conceivable that if crucial pro-inflammatory cytokines displayed highly positive charges instead of the observed negative charges, our immune system could be compromised due to the entrapment of these cytokines within ECM resulting in ineffective initiation of immune responses. Thus**, we propose a novel hypothesis that cytokine charge plays a critical role in regulating biodistribution and activity, contributing to immune regulation in response to the physiological and immunopathological microenvironment.** To test this hypothesis, we review whether there is indeed a trend of charge disparity across different types of cytokines, which may provide a novel insight in immune regulation and lay out the foundation for the further investigation. In this review, we took a comprehensive survey of cytokine charge across different species (human, mouse, and rat) based on theoretical predication and measured isoelectric points (pIs). We further discussed the link between cytokine charge disparity and in vivo biodistribution, circulating time, and spatiotemporal activities in regulating the immune status in different diseases with acidosis.

2. Cytokine charge: an evaluation of protein isoelectric point

Isoelectric point (pI) refers to a specific pH that a protein has a zero net charge. Proteins with a pI lower than 7.4 display negative charges at physiological pH, which increases the diffusion dynamics within the physiological microenvironment. Conversely, cytokines with a pI greater than 7.4 have a positive charge and tend to bind to the negatively charged ECM and serum proteins. Predicted pIs from different programs may vary due to the different algorithm used in estimating the pKa of ionizable amino acids [35]. In addition, different protein folding results in protein isomers and conformers [36], which give rise to different pI in isoelectric focusing measurements [37]. Some cytokines may also have post-translational modifications (PTM), e.g. enzyme cleavage, phosphorylation, glycosylation, etc., which may alter the pI in experimental measurements [38,39]. An online Proteome Isoelectric Point Database includes pI predictions utilizing over twenty different algorithms [40]. As shown in Table 1, we have extracted the mean and standard deviation of predicted pIs via all twenty programs for key cytokines. A comprehensive evaluation of different pI prediction algorithms revealed that the Bjellquivst algorithm with Expasy pKa set [41] achieved the best pI predictions with an optimal combination of correlation coefficient and RMSD on a set of benchmark proteins with measured pIs [42]. Thus, we have also listed the predicted pI based on Bjellquivst algorithm in Table 1, which are available on an online Phosphosite database [43]. We have also summarized the experimental pI for some recombinant cytokines that are available in the literature and included the pI of mouse and rat cytokines to examine the charge disparity in cytokines across species in Table 1.

2.1. Cytokine charge disparity

The predicted pIs are listed from low to high in Table 1 and cytokines are color-labeled to denote their different functions as generally indicated in the literature, e.g. pro-inflammatory (red), anti-inflammatory

Table 1
Isoelectric Points of Cytokines. [39,43,48–68].

PIs from different species / Cytokine*	Human			Mouse		Rat	
	Predicted		Observed	Predicted		Predicted	
	Bjellqvist [43]	Mean±SD [48]	Recombinants	Bjellqvist [43]	Mean±SD [48]	Bjellqvist [43]	Mean±SD [48]
IL-36ra	3.87	3.39±0.82	/	5.02	4.81±0.30	/	/
IL-18	4.54	4.39±0.14	6.3[49]	4.64	4.53±0.14	4.91	4.8±0.13
IL-1β	4.7	4.59±0.13	6.3[50]	4.56	4.44±0.13	4.62	4.5±0.13
Cleaved IL-33	4.8[51]	/	/	/	/	/	/
IL-38	4.94	4.83±0.13	4.56 [52]	5.7	5.68±0.16	5.08	4.90±0.36
IL-1α	5.04	4.94±0.12	5.0[53]	5.12	5.03±0.13	5.47	5.44±0.12
IL-36 gamma	5.06	4.91±0.14	/	6.3	7.21±0.32	7.08	7.17±0.33
IL-15	5.13	5.06±0.11	/	5.16	5.08±0.12	5.14	5.07±0.11
IL-32	5.14	5.05±0.12	/	/	/	/	/
GM-CSF	5.21	5.08±0.12	/	5.84	5.85±0.18	6.11	6.2±0.23
IFNα1	5.32	5.2±0.12	5.35[54]	*9.03*	8.54±0.51	8.13	7.64±0.48
G-CSF	5.61	5.69±0.2	6.8[39]	*8.95*	8.45± 0.47	7.15	7.24±0.32
HMGB1	5.62	5.54±0.13	/	5.62	5.54±0.13	5.62	5.54±0.13
IL-1ra	5.83	5.87±0.21	4.99[55]	5.83	5.89±0.2	6.42	5.44±0.12
TNFα	5.89[56]	6.55±0.26	5.3[57]	5.01	4.9±0.12	5.44	5.05±0.12
IL-36α	5.89	5.98±0.21	/	6.3	6.38±0.27	6.11[48] (*rat IL-1*)[58]	6.13±0.31
BAFF	5.92	5.95±0.16	/	6.1	6.18±0.23	7.67	7.5±0.38
IL-23	6.02	6.15±0.26	/	5.84	5.94±0.22	5.63	5.7±0.19
IL-37	6.09	6.17±0.25	/	/	/	/	/
IL-6	6.17	6.25±0.21	5.3[59]	6.96	7.06±0.33	*8.23*	7.77±0.52
IL-27	6.18	6.33±0.24	/	*5.4*	5.38±0.12	/	/
IL-12a	6.21	6.3±0.26	/	*8.4*	7.86±0.49	*8.11*	7.66±0.46
IL-22	7.65	7.47±0.43	7.88[60]	8.27	7.78±0.54	/	/
IL-2	7.67	7.51±0.44	7.7[61]	*4.88*	4.78±0.13	6.29	6.4±0.25
IL-5	7.81	7.77±0.44	/	8.91	8.51±0.49	7.71	7.55±0.43
IL-10	8.19	7.72±0.5	8.2[62]	8.45	7.9±0.51	8.8	8.27±0.52
IL-13	8.69	8.15±0.52	/	8.34	7.86±0.5	8.34	7.84±0.51
IL-3	8.69	8.35±0.52	7.8/7.4/7.1[63]	8.39	7.94±0.5	7.65	7.47±0.44
IL-17A[64]	8.82	8.29±0.48	/	9.19	8.76±0.48	8.93	8.37±0.52
TGFβ1	8.83	8.28±0.49	9.5[65]	8.9	8.76±0.48	8.83	8.27±0.49
IL-7	8.87	8.39±0.54	/	8.88	8.4±0.54	9.04	8.64±0.53
IL-33	8.89	8.45±0.5	/	8.83	8.37±0.5	8.76	8.19±0.54
IL-9	8.93	8.32±0.56	/	8.99	8.41±0.55	/	/
IFNβ	8.93	8.53±0.43	7.7/8.2[66]	9.69	9.61±0.58	9.73	9.65±0.57
IL-4	9.17	8.76±0.49	/	8.18	7.68±0.5	8.94	8.35±0.55
IFNγ	9.5	9.45±0.63	9.95[67]	8.72	8.25±0.5	9.41	9.31±0.59
IL-36β	9.6	9.54±0.7	/	8.99	8.6±0.47	*6.42* [48] *(rat IL-1)* [58]	6.42±0.58
APRIL	9.67	9.56±0.47	/	10.06	10.02±0.56	10.2	10.18±0.56
IL-11	10.64	10.56±0.64	11.7[68]	10.9	10.8±0.68	10.9	10.8±0.68

Note: Red indicates cytokines with major pro-inflammatory function, blue indicates cytokines with major anti-inflammatory function, and green indicates cytokines with dual functions or for immune cell homeostasis. The mean±SD values of cytokines were calculated based on the prediction by twenty algorithm assembled in the online database [48]. These algorithum are: Bjellqvist, DTASelect, Dawson, EMBOSS, Grimsley, IPC2_peptide, IPC2_protein, IPC_peptide, IPC_protein, Lehninger, Nozaki, ProMoST, Rodwell, Sillero, Solomon, Thurlkill, Toseland, Wikipedia, IPC2.protein.svr19, IPC2.peptide.svr19.

(blue) and dual functional (green) [44]. It is clear in Table 1 that the majority of pro-inflammatory cytokines are negatively charged and located at the top section of the table with a pI less than 7.4. On the other hand, the majority of anti-inflammatory cytokines are positively charged with pIs greater than 7.4 as shown in the bottom part of the table. Major cytokines in both mouse and rat display the same charge disparity for pro- vs anti-inflammatory cytokines. Only a few animal cytokines were observed to have opposite charges compared to human, i.e. IFNα, GM-CSF, IL 27, and IL-12a for mouse and IL-6, IL-12a, and IL-36b for rat.

We plotted all the predicted and measured pIs in Table 1 as a dot-plot in Fig. 1. As shown in Fig. 1, pro-inflammatory cytokines are mostly negatively charged with pI < 7.4, while anti-inflammatory cytokines are mostly positively charged with pI > 7.4 at physiological pH. Pro-inflammatory cytokines have a median pI of 5.9 while anti-inflammatory cytokines have a median pI of 8.2 (Fig. 1). Cytokines with dual functions, e.g., IFNα, IFNβ, IL-27, IL-12a, BAFF, APRIL and IL-2, have either positive or negative charges with mean pI of 6.2 (Fig. 1). Of note, IL-6 (pI of 6.17) is also considered a dual functional cytokine with mainly pro-inflammatory but also anti-inflammatory effects [45,46]. Recombinant cytokines have been applied as therapeutics in disease treatments [47]. Generally, the pIs of these recombinant cytokines have been measured via electrophoresis focusing assays. As shown in Fig. 1, there is no consistent trend of increase or decrease for measured pIs in

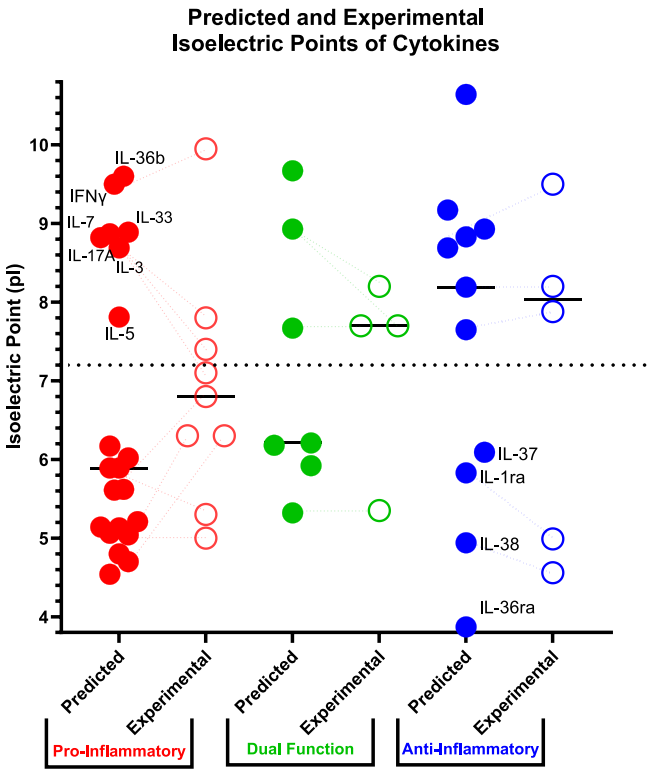


Fig. 1. Isoelectric Point of Pro- vs Anti-inflammatory Cytokines. Median pI: Pro-Inflammatory – Predicted: 5.9, Experimental: 6.80; Dual Function – Predicted: 6.2, Experimental: 7.7; Anti-Inflammatory – Predicted: 8.2, Experimental: 8.0.

comparison with predicted PI due to the different recombinant sequences. It is important to note that the measured pIs still demonstrate the charge disparity for pro- and anti-inflammatory cytokines.

2.2. *Exceptions for charge disparity*

Although significant charge disparity is observed between different group of cytokines, six out of twenty (6/20) surveyed pro-inflammatory cytokines have significant positive charges (pI > 7.4), i.e., IFNγ, IL-36β, IL-17A, IL-3, IL-7, and IL-33. Similarly, four out of eleven (4/11) anti-inflammatory cytokines have significant negative charges, i.e., IL-1ra, IL-37, IL-38, and IL-36ra (Fig. 1).

Cytokines that regulate innate immunity are produced primarily by mononuclear phagocytes such as macrophages and dendritic cells in response to PAMPs, e.g. LPS, although they can also be produced by T-lymphocytes, NK cells, endothelial cells, and mucosal epithelial cells. Cytokines produced in response to PRRs on cell surfaces, such as the inflammatory cytokines IL-1β, IL-1α, IL-6, IL-8, G-CSF, GM-CSF and TNFα, mainly act on leukocytes and endothelial cells in order to promote and control early inflammatory responses [69]. These key pro-inflammatory cytokines involved in the innate immunity mostly adopt negative surface charges (pI < 7.4) (Table 1) to ensure free diffusion to send inflammatory signals remotely for effective recruitment and activation of immune cells. However, several IL-1 family cytokines have opposite charges against this trend, including positively charged pro-inflammatory IL-33, IL-36β and negatively charged anti-inflammatory IL-1ra, IL-36ra, IL-37 and IL-38. These "outliers" may be attributed to their specific activation process, different working mechanisms, and specific microenvironment for immune functions, which are deciphered as following.

2.2.1. *"Outliers" in proinflammatory cytokines*

Interleukin-33 (IL-33) is a tissue-derived nuclear cytokine from the IL-1 family abundantly expressed in endothelial cells, epithelial cells, and fibroblast-like cells during both homeostasis and inflammation [70, 71]. It functions as an alarm signal (alarmin) released upon cell injury or tissue damage to alert immune cells expressing the ST2 receptor (IL-1RL1) and plays a key role in innate activation of allergic inflammation. Intracellular full sequence of IL-33 is positively charged with a nuclear sequence with a pI of 8.89. After leaking into extracellular space during cell necrosis, it is cleaved by neutrophil enzyme into its active immune stimulating form with a pI ~4.8 [51] (Table 1), which follows the trend of charge disparity.

IL-36 family belongs to a larger IL-1 superfamily and consists of three agonists (IL-36α/β/γ), one antagonist (IL-36Ra), one cognate receptor (IL-36R), and one accessory protein (IL-1RAcP) [72]. As shown in Table 1, most IL-36 cytokines have negative charges, except IL-36β with of high pI of 9.6. Neutrophile-derived cathepsin G cleavage of IL-36β is needed for activation of potent pro-inflammatory reactions [73]. The IL-36 interleukins are most active in barrier tissues, such as the skin, lung, and intestines, suggesting that their main responsibility is to regulate the interaction between the environment and the body [74]. In the skin, IL-36 contributes to host defense through promoting the inflammatory response. The positively charged IL-36β has a R/K rich sequence at C terminal (RKKWKSSFQHHHLRKKDKD), which may bind to heparin in ECM to prolong its tissue residency for effective enzymatic activation to induce strong local inflammation.

Interferons, which play a critical role in activation of inflammatory pathways to induce immune response upon viral infection to control dissemination including at mucosal sites [75], do not all follow this trend and have a positive charge. IFNγ, known as the only member of the type II interferons, is a pleiotropic cytokine with antiviral, antitumor, immunoregulatory, and antiproliferative activity [76]. IFNγ has a significant positively-charged heparin binding domain [77], which yields overall significant positive charges. Heparin and heparan sulfate binding regulate the biodistribution, stability, and activity of IFNγ in physiological and pathological processes [78] via preventing enzyme degradation [79] and blocking interferon receptor-binding sites to regulate its activity [80].

Cytokines in adaptive immunity. Adaptive immunity is a slower-acting, longer-lasting, and more specific response than the innate response. Key pro-inflammatory cytokines in adaptive immunity, including IL-3, IL-5, IL-7, IL-17A and IL-12a, are primarily produced at sites of infection/inflammation in tissues by T- and B-lymphocytes to eliminate pathogens and control infection [81]. Unlike the major pro-inflammatory cytokines involved in innate immunity, those in adaptive immunity do not follow the trend but instead have a pI> 7.4 and are thus positively charged (Table 1, Fig. 1). One possible explanation is that these cytokines primarily exert their function locally and thus the positive charge contributes to interactions with negative ECM proteins and heparin for their retention at the site of inflammation in tissues for longer effects to slowly acquire protective immunity.

2.2.2. *"Outliers" in anti-inflammatory cytokines*

Cytokines that regulate and suppress the inflammatory response act by two primary mechanisms: 1) active function via receptor binding to signal anti-inflammatory pathways or 2) passive inhibition of pro-inflammatory cytokines via competitive antagonism of pro-inflammatory receptors. Soluble cytokine receptors directly bind to cytokines to block intracellular signal transduction, and thus neutralize cytokine function. Most active anti-inflammatory cytokines (IL-4, IL-10, IL-11, IL-13, TGFβ), which exert their anti-inflammatory function via receptor binding and signal activation, follow the trend with positive charges (pI>7.4). However, the passive anti-inflammatory cytokines primarily consisting IL-1 family antagonists, e.g. IL36ra [72], IL-1ra [82], IL-37 [83], and IL-38 [84] (Fig. 1), antagonize their pro-inflammatory cytokine counterparts through competitive receptor binding and thus have similar chemical structures with the pro-inflammatory IL-1 family cytokines including possessing negative

charges.

3. Homogenous chemokine charges

Chemokines are a subset of cytokines that primarily serve to regulate cell migration, particularly of leukocytes, to recruit the immune response to tissues and organs [85]. Other important functions of chemokines include promoting helper T cell differentiation and blood vessel formation [86]. Chemokine structures are characterized by various cysteine residue patterns in the primary protein sequence that form disulfide bonds to establish the protein structure [87]. Chemokine activity is highly dependent on their immobilization onto cell surfaces and the extracellular matrix (ECM), which is primarily mediated by negatively charged glycosaminoglycans (GAGs) such as heparins [88]. It is well known that most chemokines (44 out of 48 as shown in Table 2) are positively charged with pI ranging between 8.49–10.97. Several of the most well studied potent pro-inflammatory chemokines, e.g. and MCP-1/2/3/4 (CCL2/8/7/13) and human IL-8 (CXCL8), have positive charges with pI ranging from 9.06–9.72. (Table 2). The positive charges of chemokines are critical for their interactions with negatively charged GAGs to establish concentration gradients to promote immune cell chemotaxis towards the site of infection and inflammation. Although chemokines can be further classified as inflammatory or homeostatic, there is no charge disparity between these two groups as all of these chemokines possess significant positively charged sequence for GAG binding [89].

Notably, only four (CCL3, CCL4, CXCL15, and CX3CL1) out of 48 chemokines surveyed do not follow this pattern and have a pI less than 7.4 (negatively charged). This is likely due to the specific cell sources for production and functionality of these chemokines. **CCL3 and CCL4**, also called macrophage inflammatory proteins, are produced by activated human monocytes, neutrophils, and lymphocytes in the peripheral blood. Of 28 CCL chemokines, only CCL3 and CCL4 are highly acidic and secreted as heterodimer that can form high molecular weight polymers under physiological relevant concentrations [91]. Aggregation of these chemokines protects against enzymatic degradation and sustains the tissue presence even without GAG binding [92]. The aggregates dynamically release chemokine monomers for chemoattractant and immune modulation [91]. In addition, these two chemokines have anti-HIV effects via competitive CCR binding [92].

CXCL15 is a mouse homolog of IL-8 [90], and also called Lungkine. It primarily serves as an important mediator of neutrophil migration from the lung parenchyma into the airspace during pulmonary inflammation [93]. It also serves as a negative regulator of multiple hematopoietic progenitor cells [94]. Unlike other CXCL chemokines, CXCL15 protein structure contains an extended C-terminal domain of ~65 aa, which contributes to an overall low isoelectric point and renders novel functions of hematopoietic regulation in addition to chemotactic properties [95].

CX3CL1 is a transmembrane protein that acts as an adhesion molecule with high molecular weight of 42 kDa [96] and can be cleaved into solution as a mature chemokine. N terminal AA1-AA76 has CX3C motif for cytokine functions, and soluble form CX3CL1 possesses a long mucin like domain from AA77 to AA317 [97], which contribute to the negative charges of the cytokine. It also promotes neo-angiogenesis and the migration of vascular smooth muscle cells [98], and thus plays an important role in vasculitis pathogenesis [99] and regulation of tumor cell invasion [100].

4. Post translational modifications (PTM) of cytokines

PTM processes have a significant impact on the structure and function of proteins [101]. PTMs, e.g. phosphorylation, sulfonation, acetylation, lactylation, deamination, and hydrophobic modifications, can also alter the charge properties, most often causing a decrease in the pI of proteins through addition of a negative group or blocking a positive group on proteins. PTMs are important mechanisms that regulate the availability and activity of cytokines and chemokines [102]. During inflammation, the upregulation of protein-modifying enzymes such as matrix metalloproteases (MMPs), plasmin, CD13, CD26, and peptidylarginine deaminases and agents like peroxynitrite may lead to the truncation, degradation, nitration, or citrullination of chemokines. This may further alter their biological activity [102]. Proteolysis via specific enzyme cleavage plays the most important role in the activation and regulation of cytokines and chemokines in inflammation and cancer [103,104]. In most cases, IL-1 family members, especially the pro-inflammatory cytokines IL-1α, IL-1β, IL-18, IL-33, IL-36α, IL-36β, IL-36γ, are subjected to proteolytic processing intracellularly or after secretion to convert into mature bioactive forms [103]. Of note, positively charged IL-33 is cleaved by an enzyme produced by neutrophils into its active immune stimulating form with reduced pI of 4.8 to result in a negative charge [51]. Interestingly, negatively charged anti-inflammatory IL-1 family cytokines, i.e. IL36ra, IL-1ra, IL-37, and IL-38, are primarily in active forms and do not require further proteolysis [103]. Most cytokines are glycosylated in the mature state in cells as N-linked or O-linked forms [105], e.g. IL-6 [106] and IL12 family cytokines [107], to regulate their activities. Glycosylation can enhance or inhibit cytokine stability and receptor binding activity [108]. Glycosylation may also further increase the negative charges of cytokines via introducing sialic acid residues, and thus may respond to the acidosis in inflammation.

Chemokines also undergo PTMs to fine-tune their biodistribution and activity [102]. Interestingly, most chemokines are not subject to glycosylation according to bioinformatics predictions [109]. Chemokines interact with G protein coupled receptors (GPCRs) through a two-step, two site process: after initial recognition and binding, the N-terminus of the chemokine inserts into the GPCR pocket to alter receptor conformation and activate intracellular signals. These

Table 2
Isoelectric points of human chemokines.

Category	Chemokines	pI [43]
Chemokine Subfamilies With Positive Charges	CCL -1, −5, −6, −9, −10, −11, −12, −14, −15, −16, −17, −18, −19, −20, −21, −22, −23, −24, −25, −26, −27, −28	8.49-10.23
	CXCL -1, −2, −3, −4, −5, −6, −7, −9, −10, −11, −12, −13, −14, −15, −16, −17	8.93-10.97
	XCL -1, −2	10.22-10.62
Key Chemokines with positive Charges	CCL2 (MCP-1)	9.4
	CCL8(MCP-2)	9.47
	CCL7(MCP-3)	9.72
	CCL13(MCP-4)	9.06
	CXCL8 (human IL-8)	9.1
Special Chemokines with Negative Charges	CCL3 (MIP-1a)	4.77
	CCL4 (MIP-1b)	5.13
	CXCL15 (mouse IL8) [90]	6.89
	CX3CL1	6.08

interactions may be perturbed by the modification of residues on chemokines, especially for N-terminal processing [110]. The effect of enzymatic cleavage is highly complex. Depending on the chemokine and the type of truncation, cleavage can result in either an increase or a decrease in biological activity [111]. N-terminal cleavage of chemokines by CD26 (Dipeptidyl peptidase IV) or matrix metalloproteases (MMPs) has little effect on their overall charge. The N-terminal cleaved chemokine remains positively charged to associate with GAGs to either potentiate or antagonize immune cell chemotaxis [112]. In contrast, C-terminal processing of chemokines by MMPs mainly affects their capacity to bind to GAGs, resulting in degradation and inactivation, such as in the case of CXCL11 cleaved at its C-terminal helix by MMP-8 and MMP-12 [113]. In summary, the significance of charge disparity and charge effects for both cytokines and chemokines are generally sustained in PTMs for immune regulation.

5. Charge effects of cytokines on ECM binding

The extracellular matrix (ECM) plays a critical role in regulating the distribution of many proteins, ions, and drugs [114], as well as serving as an important barrier to infectious agents [115]. Size and charge selective properties of the ECM control the distribution of molecules within the extracellular space. For molecules smaller than the mesh size of the ECM, e.g. signaling molecules, surface charge significantly contributes to interactions with the ECM and thus molecule distribution [115]. Heparan sulfates are thought to be of particular importance to the barrier function of the ECM as they form localized charged patches to allow for unspecific but strongly selective filtering of particles in the extracellular environment [115,116]. Heparin and heparin sulfates have an average of 2.7 and about 2 negative charges per unit, respectively, due to the presence of sulfo- and carboxyl groups. Heparin-protein binding is predominantly characterized by clusters of positively charged basic amino acid clusters in proteins interacting with negatively charged sulfo- or carboxyl groups of the glycosaminoglycan (GAG) chain in heparin molecules [117]. Clusters of basic amino acids that form heparin binding sites in proteins can occur in the primary amino acid sequence, however, clusters may also be formed as a result of higher order protein structure resulting in basic amino acids close in space but not necessarily in amino acid sequence [118]. Heparin binding sites frequently contain clusters of 1, 2, or 3 basic amino acids flanked by 1 or 2 non-basic residues on both sides [119]. Of the basic amino acids, arginine and lysine are most found at heparin and heparin sulfate-binding sites. Arginine binds heparin about 2.5x tighter than lysine at physiological pH with more stable hydrogen bonds and electrostatic interactions [120]. However, higher heparin binding affinity can be detrimental for in vivo chemotactic effects and lysine residue fine-tunes in vivo chemokine-GAG interactions and chemotaxis function [121].

As discussed above, chemokines bind strongly to GAGs in ECM to recruit immune cells for immune modulation. It is also known that numerous growth factors and cytokines, especially positively charged cytokines as shown in Table 1, e.g. IL-10 [122], TGF-β [123], IL-4 [124], IL-8 [125], IL-7 [126] and IFN-r [127], etc., bind to heparan sulfate and heparin in ECM to locally regulate inflammation and promote tissue repair. ECM proteins and heparan sulfates interact with these proteins to regulate their storage and activity [128]. Also, cell-surface GAGs with sulfate groups are important in binding and modulation of IL-10 activity [122]. Highly positively charged rhIFN-γ was found to bind heparin-agarose consistently at pH from 4 to 9, whereas, negatively charged rhIL-1β can't bind heparin-agarose beads at these pH ranges [129]. Additionally, several other cytokines e.g. rhIL-4, rhIL-2, and TNF-α only show strong heparin binding at pH 5. These studies provide the evidence for selective cytokine heparin binding based on the charge disparity.

6. Cytokine pharmacokinetics (PK) and metabolism

Immune homeostasis is closely regulated locally and systemically. Production of cytokines is orchestrated to regulate the immune response for effective immune defense. The elimination of cytokines via enzymatic degradation, liver metabolism, and renal clearance after diffusion from tissue into blood also plays an important role to control inflammation for timely resolution. Numerous recombinant cytokines have been developed for disease treatments. The characterization of the in vivo pharmacokinetics of the exogenous cytokines provides insight for better understanding of the mechanism of endogenous immune regulation. In addition to receptor binding and enzyme cleavage, the tissue/ECM affinity, plasma protein association, and renal filtration of cytokines also contribute to their elimination [130]. These elimination processes are determined by their general physiochemical properties, e.g. charges, molecular weight, shape, and structural modification [131]. A comprehensive review [132] on the charge effects on antibody tissue distribution and PK profile concluded that: (I) shifts in pI of one unit or more can produce measurable changes in tissue distribution and kinetics, (II) increases in net positive charge generally result in increased tissue retention and increased blood clearance, and (III) decreases in net positive charge generally result in decreased tissue retention and increased whole body clearance. Thus, it is reasonable to believe that the charge disparities between pro-inflammatory cytokines, anti-inflammatory cytokines, and positively charged chemokines play important roles in regulating the tissue distribution and blood circulation time to control inflammation.

Renal filtration is a homeostatic process involved in the regulation small proteins and is directly proportional to the plasma concentrations of small protein to result in the renal catabolic or urinary excretion of the filtered loads [133]. Small signaling molecules, e.g. chemokine, are subjected to fast renal clearance, since therapeutic cytokine levels drop rapidly after injection [134]. A cytokine PK study revealed that the negative charged IL-6 and IL-1β can be eliminated more efficiently than the positively charged IL-10 and CXCL1 from blood after intravenous injection in normal mice, which can be impaired in mice with bilateral nephrectomy [135]. This finding may be due to the weak GAG association of the negatively charged cytokines. Although TNFα did not demonstrate efficient renal clearance in this study, this may be due to systemic inflammation and active TNFα production induced by the injection of high dose of TNFα (200 ng/mouse). The endogenous negatively charged pro-inflammatory cytokines produced in tissue have better tissue penetration for effective initiation of inflammation. At the same time, their fast elimination avoids the prolonged and overwhelming inflammation. On the contrary, the positively charged chemokines and anti-inflammatory cytokines may reside in tissue for a longer time to recruit immune cells for infection/tissue damage control, and inflammation control, respectively.

Kidney and liver metabolism are main routes for elimination of interleukins in addition to renal filtration, while other organs have a negligible role. The kidney is particularly important and eliminates a significant amount of circulating IL-1, IL-2, IL-3, and TNFα in rats [163]. The rat kidneys can also reabsorb certain interleukins, e.g. IL-2 and IL-3, when tubular cells are normal. IL-1 in the tubular fluid may be partially hydrolyzed at the border or directly absorbed. However, the human kidney may have less resorptive capacity than the rat kidney. Once absorbed, cathepsin D in lysosomes hydrolyzes interleukins and albumin. Intact proteins are not returned to circulation [138]. Interleukins can be broken down in the liver due to functional and/or clearance receptors, such as hepatic lectins. These receptors are found in hepatocytes, Kupffer cells, and endothelial cells, leading to various biological effects and/or catabolism [163]. When escalating doses of IL-2 or TNFα were given intravenously, a progressive prolongation in half-lives and a reduction in the apparent volume of distribution (Vd) were observed, indicating the importance of cell receptor binding at low doses [164]. It is known that slightly anionic macromolecules showed relatively low

Table 3
Half-Life of human recombinant cytokines in human or animal models.

Cytokines	MW (kDa)	Animal Models (Injection routes)	Half-Life (min/hr)			Ref.
			Two-compartment		Non-compartment	
			Distribution	Elimination	Elimination	
Negatively Charged Cytokines						
hrIL-1β	30.7[a]	- Mice (I.V.)	~5 min	~27 min		[136]
		- Rat (I.V.)	~3 min	~4 hr		[137]
		- Rat (I.V.)	/	/	19 min	[138]
hrTNF-**α**	25.64[a]	- Human (I.V.)	~24 min	/		[139]
	~17[b]	- Mice (I.V.)	18.5-19.2 min	102-162 min		[140]
		- Rat (I.V.)	2.1 min	14.4-31.8 min		[141]
hrIL-15	12.9[b]	- Human (I.V.)	/	/	~2.5 hr	[142]
hrIL-6	19-30[b]	- Mice (I.V.)	/	/	~7 min	[143]
		- Mice (I.P.)	/	/	~3 hr	[143]
		- Rat (I.V.)	~3 min	~55 min		[144]
hIL-12	70[a]	- Rat (I.V)	/	/	2-5 hr	[145]
	(heterodimer)	- Monkey (I.V.)	/	/	13-19 hr	[146]
hrIL-12		- Human (S.C.)	/	/	30 h	[146]
hrIL-1ra	17.3[b]	- Human (I.V.)	21 min	108 min		[147]
		- Human (I.M)	/	/	2.22 ~ 3.29 hr	[148]
		- Human (S.C.)	/	/	4~6 hr	[149]
		- Mice (I.P.)	/	/	0.5 ~ 3.2 hr[g]	[150]
hIFN α-2b	19[a]	- Human (I.V.)	0.1 hr	1.7 hr		[151]
		- Human (S.C.)	/	/	2.9 hr	[151]
		- Human (I.M.)	/	/	2.2 hr	[151]
Neutral cytokine						
hrIL-2	17.6[a]	- Human (I.V.)	6-7 min	~60 min		[152]
		- Human (I.V.)	12.9 min	85 min		[153]
		- Mice (I.V.)	/	/	7-9 min	[154]
	25.2[b]	- Mice (S.C.)	/	/	44 min	[155]
Positively Charged Cytokines						
mIL-10	35 (dimer)[b]	- Mice (I.V.)	/	/	141.12 min	[156]
hIL-10	18[b]	- Mice (I.V.)	/	/	57.6 min	[156]
hrIL-10	20.5[a]	- Human (I.V.)	/	/	2.3-3.7 hr[d]	[157]
hrIL-7	20.2[a]	- Human (S.C.)	/	/	6.46-9.8 hr[c]	[158]
hrTGF-β1	25[b]	- Rat (I.V.)	10.7 min[e]	61 min[e]		[159]
		- Rat (I.V.)	2.5 min [f]	163 min[f]		[159]
pigTGF-β1		- Rat (I.A.)	/	3.1 min		[160]
IL-4	17.5[a]	- Mice (I.V.)	/	/	19 ± 2 min	[161]
hrIL-4	15.4[b]	- Human (I.V.)	/	/	19 ± 8.7 min	[162]
hrIFN-γ	19.3[a]	- Rat (I.V.)	1.1 min	94 min		[79]

Notes: [a] predicated molecular weight of the native cytokines or [b] the reported molecular weight of the recombinant protein reported in the literature; [c] S.C. dose 10 ug/kg to 60 ug/kg (detected by ELISA); [d] I.V. dose 25 ug/kg to 100 ug/kg (detected by ELISA); [e] I.V. dose 1 mg/kg (detected by ELISA); [f] I.V. dose 50uCi/kg (detected by TCA precipitable radioactivity); [g] I.P. dose 1.5 mg/kg to 2.5 mg/kg (detected by ELISA).

hepatic clearance [165]. The pharmacokinetic studies of IL-1, −2, −3, −6, and TNFα revealed rapid cleanup from the bloodstream after iv injection [134].

In vivo catabolic pathways, such as renal filtration and liver uptake, efficiently extract these proteins from circulation. This is a beneficial mechanism as these cytokines are highly potent immunomodulators. The distribution half-life of the recombinant cytokines has been determined in minutes, whereas the elimination half-life lasts several hours. Table 3 summarizes some experimental data of in vivo serum half-life for some recombinant human cytokines as therapeutics administrated via different routes, e.g., intravenous (I.V.), intraperitoneal (I.P.), subcutaneous (S.C), or intramuscular (IM) injections in human or animal models. Generally, cytokines have very short half-life in vivo due to small molecular weight [153]. In comparison, hrIL-12 forms a heterodimer with molecular weight of 70 kDa, which significantly prolongs $t_{1/2}$ to hours in human and about a day in monkey. There is also a trend that the administration routes via subcutaneous, intramuscular, or intraperitoneal injections prolong the elimination time compared to intravenous injection. Given the limited studies with different recombinant proteins, the impact of cytokine charge on PK and biodistribution profiles is still unknown. It is important to note that cytokines are a diverse group of molecules with varying physicochemical properties, and their biodistribution and half-life can be influenced by multiple factors beyond their pI, such as receptor binding, protein size, and post-translational modifications.

7. Cytokine charge disparity in diseases with pH alteration

Inflammation is involved in the pathogenesis and progression of many diseases. Inflammatory cytokines and chemokines are implicated in regulating both immune cells and tissue cells in the diseases. In particular, pH alterations that occur in certain pathological microenvironments, e.g., solid tumor, chronic wound healing and infections, potentially have a direct impact on cytokine activity through the charge disparity to regulate the immune response and promote pathogenesis.

7.1. Acidosis in solid tumors

Inflammation is a hallmark of cancer and participates in all cancer stages, i.e., tumorigenesis, progression, metastasis, and cancer therapy [166]. Inherently, cytokines play an important and pleiotropic role in both the promotion and inhibition of cancer tumors [167]. For instance, IL-22 has shown to regulate the expression of many genes and activate DNA-damaging responses in epithelial stem cells to prevent mutagenesis and carcinogenesis [168]. During tumor progression and metastasis, NF-kB controls the expression of more than 100 inflammatory genes including cytokines (TNFα, IL-6, IL-1β, etc.) that promote cancer growth, epithelial-mesenchymal transition, and migration [169]. In addition, cytokines such as IL-8 and VEGF play a key role in cancer angiogenesis, invasion, and metastasis [170,171]. However, other cytokines can have anti-tumor activity, such as IFN γ, IFNα, IL-2, and IL-12, to stimulate

anti-tumor NK cells or cytotoxic T cells in both innate and adaptive immunity [167,172].

Cancer growth dramatically upregulates aerobic glycolysis resulting in an excessive amount of lactic acid production and acidification of tumor microenvironment, known as Warburg effect, to adapt to hypoxic conditions [173]. Solid tumor acidosis ranging from pH 6 to 7 can disrupt immune function resulting in worsened immune surveillance [9]. Extensive reviews have been published on the immunosuppressive and pro-tumor effects of tumor acidosis on immune cell functions in the local microenvironment [174]. For example, lactic acidosis was found to upregulate the expression of TGFβ, which in turn regulates VEGF and MMPs expression, reinforcing the cancer aggressiveness [175]. Additionally, it was found that acidic pH decreases natural killer and T cells in colorectal cancer and that pH modulation decreases levels of cytokines with EMT role while increasing cytokines that activate immune cells [175]. However, there was no discussion on whether tumor acidosis also alters the activity of cytokines, e.g., decrease the potency of negatively charged pro-inflammatory cytokines and increase the potency and residency of positively charged anti-inflammatory cytokines, thus contributing to immune suppression. Recently, a study for IL-2, a cytokine crucial for CD8 T cell and NK cell function, has shown that the IL-2:IL-2Rα interaction was reduced substantially at acidic pH 6 where the cytokine receptor binding site has a pH switch region that controls the interaction. As a result, an IL-2 variant was designed that is insensitive to pH change and has an improved therapeutic effect [176]. TNFα analogs with more positively charged flexible N-terminal region demonstrated increased binding to the negative glycosaminoglycans, initiating cell surface interaction to result in more effective targeting to TNFα receptor for increased induction of cytotoxicity [177]. An improved understanding of the charge properties of cytokines and the response to the tumor acidosis may facilitate enhanced cytokine engineering to harness strong anti-cancer immunity for effective tumor treatments and immunotherapy.

7.2. Wound healing

Healthy skin surface is known to have slightly acidic pH ranging from 4 to 6 [178], which maintains the balance of skin's natural oils and defends against airborne pathogens such as bacteria. Upon skin injury, multiple cellular activities and molecular cues take place in the lesion sites, undergoing four overlapping phases: hemostasis, inflammation, proliferation, and remodeling. Acute wounds generate a rapid and strong immune response during the inflammation phase to clear the damaged cells and potential pathogen. It is known that pH reduces as acute healing advances, suggesting that skin surface pH may serve as a potential indicator for the healing stage and a target for therapeutic intervention [179]. Chronic wounds result from an insufficient ability to recruit an appropriately strong immune response, but rather maintain a persistent low-grade mild inflammation and thus fail to transform from inflammation phase to proliferation phase [180].

pH changes affect wound healing and treatment of both chronic and acute wounds through influencing the skin cell response, immune cell response, enzyme activity, microbial virulence, and drug efficacy [181]. In addition, pH changes may also interfere cytokine activities and thus contribute to the wound immune status. pH changes differ significantly in acute versus chronic wounds: acute wounds have a more neutral pH followed by a drop in pH due to hypoxic conditions and production of lactic acid, whereas delayed chronic wounds have an oscillated alkaline pH ranging 7.2 to 8.9 [179]. Bacteria present in chronic, non-healing wounds with infection can produce toxic substances, i.e., ammonia, which increases the alkalinity of the wound tissue [182]. Elevated pH levels in chronic wounds may sustain a mild level of inflammation by decreasing the activity and reducing the ECM binding of the positively charged anti-inflammatory cytokines. Additionally, the elevated pH favors certain protease activity and disrupts ECM construction, thus hampering wound healing process [182].

7.3. Infection

During bacterial infection, local acidosis can range from pH 5.9 to 7.0 due to a combination of factors including tissue hypoxia resulting in anaerobic glycolysis and accumulation of lactic acid, bacterial fatty acid production, and hypochlorous acid production by activated neutrophils at the site of infection [5,183,184]. Local acidosis has been linked to a variety of infectious disease processes including septic arthritis, urinary tract infection, peritonitis, and lung infections, proving to be protective in some instances while destructive in others. For example, in gram negative septic arthritis, high white blood cell count is strongly associated with decreased pH in the synovial fluid, and it is believed that this connection contributes to poor therapeutic response to aminoglycoside antibiotics in these patients [185]. On the other hand, extracellular acidosis in the kidney is associated with the expression of antimicrobial peptides and resistance to uropathogenic *E. coli* infection [186].

It has been well documented that acidosis significantly impacts immune cells during infection and inflammation[9]. Activation of immune cells induces a shift of energy production to aerobic glycolysis with an increase in lactate production intracellularly [3]. The resulting acidosis significantly decreases neutrophil phagocytosis for bacteria clearance and triggers the release of IL-1β as a result of immune cell pyroptosis induction to perpetuate further inflammatory reactions [187]. This results in enhanced production of pro-inflammatory cytokines and increased neutrophil recruitment to enable host clearance of the infection but can also result in collateral inflammatory damage [188]. A hypercapnic acidosis mouse model via 10% CO_2 exposure has been subjected to *Pseudomonas* pneumonia infection [189]. Acidosis was associated with increased mortality from lung infections in these mice with the decreased phagocytosis of bacteria by alveolar neutrophils. Interestingly, acidosis didn't impact neutrophil alveolar infiltration, rather it inhibited the early cytokine response (reduced TNFα and IL-6) at 7 h post infection but this effect abolished at 15 h. It indicates that immune cells may take a secondary mechanism, e.g. pyroptosis, in response to acidosis to produce IL-1β and IL-18 to boost another round of inflammatory responses for pathogen clearance upon initial failure of immune response for infection control. Unfortunately, pyroptosis and resulting IL-1β and IL-18 were not characterized in this study. It is worth mentioning that TNFα and IL-6 as early pro-inflammatory cytokines have only slightly negative charges with pIs ~6, while later cytokines IL-1β and IL-18 have much lower pIs < 5 (Table 1). Thus, TNFα may be more sensitive to the infection-induced acidosis with pH ranging from 5.9–7.0; while IL-1β and IL-18 may be less sensitive to acidosis for further effective immune stimulation under acidotic conditions.

7.4. Sepsis

Sepsis is a complex, heterogenous disease that results in a dysregulated immune response, leading to significant morbidity and mortality [190,191]. In sepsis, many immune and non-immune mediators are released, including PAMPs, resulting in a massive production of cytokines known as "cytokine storm" [191]. As a result, sepsis can cause overwhelming inflammation leading to multiple organ failure and death [192]. Like many inflammatory processes, sepsis can result in both tissue and systemic acidosis [18,19,183,186,188,193]. In sepsis, pH regulation plays a critical role in the inflammatory response. For example, a knockout model of proton sensing G protein Coupled receptor G2A resulted in decreased cytokine production, inadequate bacterial scavenging, and increased lethality in a sepsis mouse model [194]. Additionally, bicarbonate administration to restore pH balance has variable efficacy, with no survival benefit in sepsis patients with acidosis [195]. This suggests that acidosis in sepsis pathology serves as important but complex regulatory mechanism of the immune response.

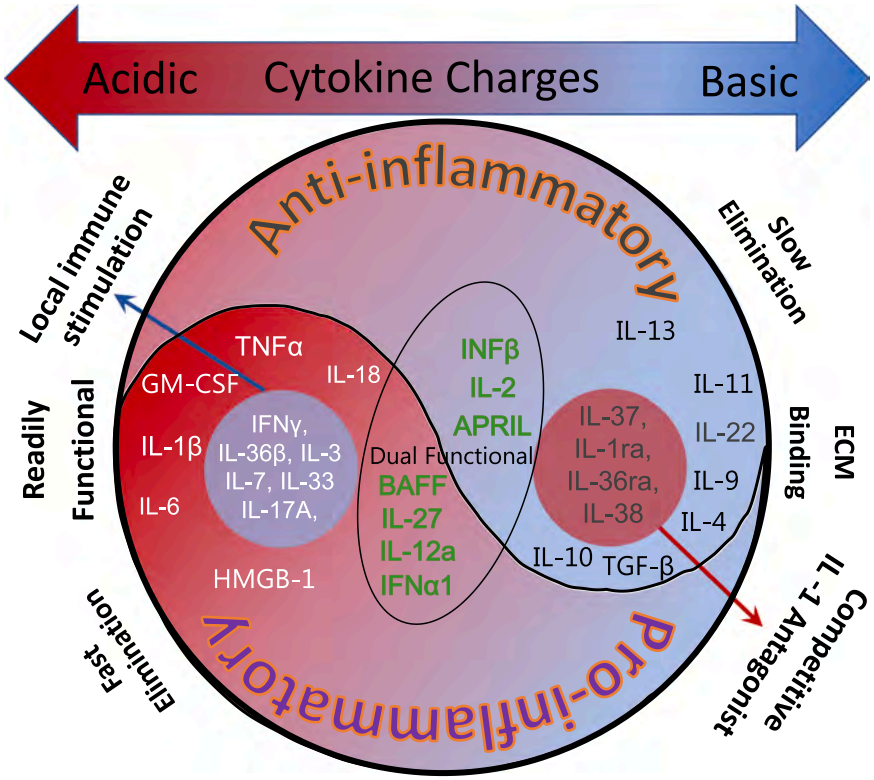


Fig. 2. Illustration of cytokine charge disparity between pro- and anti-inflammatory cytokines, which potentially regulate their bioavailability and potency according to their spatiotemporal functionality and targets.

8. Perspectives

In summary, our analysis further confirmed our initial observation of the charge disparity between pro-inflammatory and anti-inflammatory cytokines (Fig. 2). Of particular note, almost all chemokines adopt positive charges to effectively induce chemotaxis of immune cells. Although it is hard to separate the pH effects on immune cells and cytokine activity in immune regulation, it is important to recognize the charge disparity of cytokines, which provides a novel angle to explore for improved understanding of immune regulation in regular inflammation processes as well as immunopathogenesis. More importantly, we may develop effective approaches to target the charge disparity for precise immune modulation. For example, engineering of surface charges or binding site charge properties in recombinant cytokines may produce more effective therapeutics to overcome the limitation of pathogenic acidosis for disease treatments [176]. Targeting the charge disparity of cytokines may also provide a unique path to develop diagnostic and prognostic tools to precisely diagnose patient immune status, especially in sepsis given the dynamic, complex, and heterogenous cytokine and cellular dynamics. Further, more therapeutic interventions can be developed targeting the charge disparity of cytokines to modulate immune status more effectively and precisely without causing immune suppression. Our group has developed a well-defined telodendrimer nanotrap platform [196,197], which effectively captures proteins, cytokines, and endotoxin based on the synergistic combination of multiple charge and hydrophobic interactions. We have demonstrated that we can effectively capture cytokines with similar charges by introducing the opposite charges in the nanotrap to target a broad spectrum of pro-inflammatory or anti-inflammatory cytokines for precise control of hyperinflammation or immune suppression in sepsis and other critical illness [32,198].

CRediT authorship contribution statement

JM, ML, MH, HG, XY, AJ, JW, JL preliminarily collected literatures, analyzed data, generated figures/tables, and finished the manuscript. JL constructed the conceptual framework, acquired funding, supervise the work, finalize manuscript. All authors agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This work was supported by NIH/NIGMS R01GM130941 and R35GM147560, DoD PRMRP/TTD award PR221368, New York Fund for Innovation in Research and Scientific Talent (FIRST), Maureen T. O'Hara TEAL THERE's A CURE and Christine Schoeck Blakely Ovarian Cancer Research Foundation.

References

[1] O. Takeuchi, S. Akira, Pattern recognition receptors and inflammation, Cell 140 (6) (2010) 805–820.
[2] L. Chen, H. Deng, H. Cui, J. Fang, Z. Zuo, J. Deng, Y. Li, X. Wang, L. Zhao, Inflammatory responses and inflammation-associated diseases in organs, Oncotarget 9 (6) (2018) 7204–7218.
[3] C. Manosalva, J. Quiroga, A.I. Hidalgo, P. Alarcón, N. Anseoleaga, M.A. Hidalgo, R.A. Burgos, Role of lactate in inflammatory processes: friend or foe, Front Immunol. 12 (2021) 808799.
[4] X. Chen, Y. Qian, S. Wu, The Warburg effect: evolving interpretations of an established concept, Free Radic. Biol. Med. 79 (2015) 253–263.
[5] A. Lardner, The effects of extracellular pH on immune function, J. Leukoc. Biol. 69 (2001) 522–530.

[6] B.S. Ashby, pH Studies in human malignant tumours, Lancet 288 (7458) (1966) 312–315.
[7] B. Mansson, P. Geborek, T. Saxne, S. Bjornsson, Cytidine deaminase activity in synovial fluid of patients with rheumatoid arthritis: relation to lactoferrin, acidosis, and cartilage proteoglycan release, Ann. Rheum. Dis. 49 (8) (1990), 594-594.
[8] H.-P. Simmen, J. Blaser, Analysis of pH and pO2 in abscesses, peritoneal fluid, and drainage fluid in the presence or absence of bacterial infection during and after abdominal surgery, Am. J. Surg. 166 (1) (1993) 24–27.
[9] F. Erra Díaz, E. Dantas, J. Geffner, Unravelling the interplay between extracellular acidosis and immune cells, Mediat. Inflamm. 2018 (2018) 1218297.
[10] A.S. Trevani, G. Andonegui, M. Giordano, D.H. López, R. Gamberale, F. Minucci, J.R. Geffner, Extracellular acidification induces human neutrophil activation, J. Immunol. 162 (8) (1999) 4849–4857.
[11] O.D. Rotstein, V.D. Fiegel, R.L. Simmons, D.R. Knighton, The deleterious effect of reduced pH and hypoxia on neutrophil migration in vitro, J. Surg. Res 45 (3) (1988) 298–303.
[12] N. Murata, C. Mogi, M. Tobo, T. Nakakura, K. Sato, H. Tomura, F. Okajima, Inhibition of superoxide anion production by extracellular acidification in neutrophils, Cell Immunol. 259 (1) (2009) 21–26.
[13] D. El Kebir, E. de Oliveira Lima dos Santos, S. Mansouri, M. Sekheri, J.G. Filep, Mild acidosis delays neutrophil apoptosis via multiple signaling pathways and acts in concert with inflammatory mediators, J. Leukoc. Biol. 102 (6) (2017) 1389–1400.
[14] B.K. Pliyev, A.B. Sumarokov, L.I. Buriachkovskaia, M. Menshikov, Extracellular acidosis promotes neutrophil transdifferentiation to MHC class II-expressing cells, Cell. Immunol. 271 (2) (2011) 214–218.
[15] M. Behnen, S. Möller, A. Brozek, M. Klinger, T. Laskay, Extracellular acidification inhibits the ROS-dependent formation of neutrophil extracellular traps, Front. Immunol. 8 (2017) 184.
[16] C.C. Jancic, M. Cabrini, M.L. Gabelloni, C. Rodríguez Rodrigues, G. Salamone, A. S. Trevani, J. Geffner, Low extracellular pH stimulates the production of IL-1β by human monocytes, Cytokine 57 (2) (2012) 258–268.
[17] K. Rajamäki, T. Nordström, K. Nurmi, K.E. Åkerman, P.T. Kovanen, K. Öörni, K. K. Eklund, Extracellular acidosis is a novel danger signal alerting innate immunity via the NLRP3 inflammasome, J. Biol. Chem. 288 (19) (2013) 13410–13419.
[18] A. Riemann, H. Wußling, H. Loppnow, H. Fu, S. Reime, O. Thews, Acidosis differently modulates the inflammatory program in monocytes and macrophages, Biochim. Et. Biophys. Acta (BBA) - Mol. Basis Dis. 1862 (1) (2016) 72–81.
[19] J.A. Kellum, M. Song, J. Li, Lactic and hydrochloric acids induce different patterns of inflammatory response in LPS-stimulated RAW 264.7 cells, American Journal of Physiology - Regulatory Integrative and Comparative Physiology 286 (4 55–4) (2004) 686–692.
[20] N. Wang, K.L. Gates, H. Trejo, S. Favoreto, R.P. Schleimer, J.I. Sznajder, G. J. Beitel, P.H.S. Sporn, Elevated CO 2 selectively inhibits interleukin-6 and tumor necrosis factor expression and decreases phagocytosis in the macrophage, FASEB J. 24 (7) (2010) 2178–2190.
[21] M. Vermeulen, M. Giordano, A.S. Trevani, C. Sedlik, R. Gamberale, P. Fernández-Calotti, G. Salamone, S. Raiden, J. Sanjurjo, J.R. Geffner, Acidosis improves uptake of antigens and MHC class I-restricted presentation by dendritic cells, J. Immunol. 172 (5) (2004) 3196–3204.
[22] D. Martínez, M. Vermeulen, E. von Euw, J. Sabatté, J. Maggíni, A. Ceballos, A. Trevani, K. Nahmod, G. Salamone, M. Barrio, M. Giordano, S. Amigorena, J. Geffner, Extracellular acidosis triggers the maturation of human dendritic cells and the production of IL-12, J. Immunol. 179 (3) (2007) 1950–1959.
[23] E. Gottfried, L.A. Kunz-Schughart, S. Ebner, W. Mueller-Klieser, S. Hoves, R. Andreesen, A. Mackensen, M. Kreutz, Tumor-derived lactic acid modulates dendritic cell activation and antigen expression, Blood 107 (5) (2006) 2013–2021.
[24] A. Nasi, T. Fekete, A. Krishnamurthy, S. Snowden, E. Rajnavölgyi, A.I. Catrina, C. E. Wheelock, N. Vivar, B. Rethi, Dendritic cell reprogramming by endogenously produced lactic acid, J. Immunol. 191 (6) (2013) 3090–3099.
[25] Z. Husain, Y. Huang, P. Seth, V.P. Sukhatme, Tumor-derived lactate modifies antitumor immune response: effect on myeloid-derived suppressor cells and NK cells, J. Immunol. 191 (3) (2013) 1486–1495.
[26] L.H. Lv, J.D. Yu, G.L. Li, T.Z. Long, W. Zhang, Y.J. Chen, J. Min, Y.L. Wan, Functional distinction of rat liver natural killer cells from spleen natural killer cells under normal and acidic conditions in vitro, Hepatobiliary Pancreat. Dis. Int 11 (3) (2012) 285–293.
[27] M.D. Turner, B. Nedjai, T. Hurst, D.J. Pennington, Cytokines and chemokines: At the crossroads of cell signalling and inflammatory disease, Biochim. Biophys. Acta 1843 (11) (2014) 2563–2582.
[28] F.-X. Theillet, A. Binolfi, T. Frembgen-Kesner, K. Hingorani, M. Sarkar, C. Kyne, C. Li, P.B. Crowley, L. Gierasch, G.J. Pielak, A.H. Elcock, A. Gershenson, P. Selenko, Physicochemical Properties of Cells and Their Effects on Intrinsically Disordered Proteins (IDPs), Chem. Rev. 114 (13) (2014) 6661–6714.
[29] X. Jin, M. Zhou, S. Chen, D. Li, X. Cao, B. Liu, Effects of pH alterations on stress- and aging-induced protein phase separation, Cell. Mol. Life Sci. 79 (7) (2022) 380.
[30] F.J. Link, J.Y.Y. Heng, Unraveling the impact of ph on the crystallization of pharmaceutical proteins: a case study of human insulin, Cryst. Growth Des. 22 (5) (2022) 3024–3033.
[31] P.A. Prasad, M.C. Fang, Y. Abe-Jones, C.S. Calfee, M.A. Matthay, K.N. Kangelaris, Time to Recognition of Sepsis in the Emergency Department Using Electronic Health Record Data: A Comparative Analysis of Systemic Inflammatory Response Syndrome, Sequential Organ Failure Assessment, and Quick Sequential Organ Failure Assessment, Critical Care Medicine Online First (9000).
[32] C. Shi, X. Wang, L. Wang, Q. Meng, D. Guo, L. Chen, M. Dai, G. Wang, R. Cooney, J. Luo, A nanotrap improves survival in severe sepsis by attenuating hyperinflammation, Nat. Commun. 11 (1) (2020).
[33] N.B. Janakiram, M.S. Valerio, S.M. Goldman, C.L. Dearth, The role of the inflammatory response in mediating functional recovery following composite tissue injuries, Int. J. Mol. Sci. 22 (24) (2021).
[34] A.A. Tokmakov, A. Kurotani, K.-I. Sato, Protein pI and intracellular localization, Front. Mol. Biosci. 8 (2021).
[35] Lukasz P. Kozlowski, IPC 2.0: prediction of isoelectric point and pKa dissociation constants, Nucleic Acids Res. 49 (W1) (2021) W285–W292.
[36] G.D. Rose, Reframing the protein folding problem: entropy as organizer, Biochemistry 60 (49) (2021) 3753–3761.
[37] N. Ul, Isoelectric points and conformation of proteins: I. Effect of urea on the behavior of some proteins in isoelectric focusing, Biochimica et Biophysica Acta (BBA) - Protein, Structure 229 (3) (1971) 567–581.
[38] J. Lengqvist, H. Eriksson, M. Gry, K. Uhlén, C. Björklund, B. Bjellqvist, P. J. Jakobsson, J. Lehtiö, Observed peptide pI and retention time shifts as a result of post-translational modifications in multidimensional separations using narrow-range IPG-IEF, Amino Acids 40 (2) (2011) 697–711.
[39] R. Hecht, T. Tressel, V. Goldshteyn, D. Winters, J. Wilson, T. Boone, M. Daniels, Preparative electrofocusing for industrial applications, Prep. Biochem. Biotechnol. 26 (3-4) (1996) 201–216.
[40] S. Gauci, B. van Breukelen, S.M. Lemeer, J. Krijgsveld, A.J. Heck, A versatile peptide pI calculator for phosphorylated and N-terminal acetylated peptides experimentally tested using peptide isoelectric focusing, Proteomics 8 (23-24) (2008) 4898–4906.
[41] B. Bjellqvist, G.J. Hughes, C. Pasquali, N. Paquet, F. Ravier, J.C. Sanchez, S. Frutiger, D. Hochstrasser, The focusing positions of polypeptides in immobilized pH gradients can be predicted from their amino acid sequences, Electrophoresis 14 (10) (1993) 1023–1031.
[42] E. Audain, Y. Ramos, H. Hermjakob, D. Flower, Y. Perez-Riverol, Accurate estimation of Isoelectric Point of Protein and Peptide based on Amino Acid Sequences, Bioinformatics 32 (2015).
[43] PhosphoSitePlus, Cell Signaling Technology.
[44] C.A. Dinarello, Historical insights into cytokines, Eur. J. Immunol. 37 (Suppl 1) (2007) S34–S45.
[45] P. Villar-Fincheira, F. Sanhueza-Olivares, I. Norambuena-Soto, N. Cancino-Arenas, F. Hernandez-Vargas, R. Troncoso, L. Gabrielli, M. Chiong, Role of Interleukin-6 in vascular health and disease, Front. Mol. Biosci. 8 (2021).
[46] M. Ye, M.E. Joosse, L. Liu, Y. Sun, Y. Dong, C. Cai, Z. Song, J. Zhang, S.R. Brant, M. Lazarev, X. Li, Deletion of IL-6 exacerbates colitis and induces systemic inflammation in IL-10-Deficient mice, J. Crohns Colitis 14 (6) (2020) 831–840.
[47] J. Deckers, T. Anbergen, A.M. Hokke, A. de Dreu, D.P. Schrijver, K. de Bruin, Y. C. Toner, T.J. Beldman, J.B. Spangler, T.F.A. de Greef, F. Grisoni, R. van der Meel, L.A.B. Joosten, M. Merkx, M.G. Netea, W.J.M. Mulder, Engineering cytokine therapeutics, Nat. Rev. Bioeng. 1 (4) (2023) 286–303.
[48] L.P. Kozlowski, Proteome-pI 2.0: proteome isoelectric point database update, Nucleic Acids Res. 50 (D1) (2022) D1535, d1540.
[49] M.H. Goldman, D.C. James, M. Rendall, A.P. Ison, M. Hoare, A.T. Bulll, Monitoring recombinant human interferon-gamma n-glycosylation during perfused fluidized-bed and stirred-tank batch culture of CHO cells, Biotechnol. Bioeng. 60 (5) (1998) 596–607.
[50] M. Brattsand, T. Egelrud, Purification and characterization of interleukin 1 beta from human plantar stratum corneum. Evidence of interleukin 1 beta processing in vivo not involving interleukin 1 beta convertase, CYTOKINE 10 (7) (1998) 506–513.
[51] E. Lefrançais, S. Roga, V. Gautier, A. Gonzalez-de-Peredo, B. Monsarrat, J. P. Girard, C. Cayrol, IL-33 is processed into mature bioactive forms by neutrophil elastase and cathepsin G, Proc. Natl. Acad. Sci. USA 109 (5) (2012) 1673–1678.
[52] J. Mora, A. Schlemmer, I. Wittig, F. Richter, M. Putyrski, A.-C. Frank, Y. Han, M. Jung, A. Ernst, A. Weigert, B. Brüne, Interleukin-38 is released from apoptotic cells to limit inflammatory macrophage responses, J. Mol. Cell Biol. 8 (5) (2016) 426–438.
[53] J.J. Oppenheim, E.J. Kovacs, K. Matsushima, S.K. Durum, There is more than one interleukin-1, Immunology Today 7 (2) (1986) 45–56.
[54] W.A. Carter, Interferon - evidence for subunit structure, Proc. Natl. Acad. Sci. USA 67 (2) (1970) 620.
[55] H.H. Rasmussen, J.E. Celis, Evidence for an altered protein-kinase-c (PKC) signaling pathway in psoriasis, J. Investigative Dermatology 101 (4) (1993) 560–566.
[56] L. Kozlowski, I.P.C.- Isoelectric Point Calculator, 2016. ⟨http://isoelectric.org/calculate.php⟩. (Accessed 07/28/2023.
[57] B.B. Aggarwal, W.J. Kohr, P.E. Hass, B. Moffat, S.A. Spencer, W.J. Henzel, T. S. Bringman, G.E. Nedwin, D.V. Goeddel, R.N. Harkins, Human tumor necrosis factor. Production, purification, and characterization, J. Biol. Chem. 260 (4) (1985) 2345–2354.
[58] E. Coudert, S. Gehant, E. de Castro, M. Pozzato, D. Baratin, T. Neto, C.J.A. Sigrist, N. Redaschi, A. Bridge, T.U. Consortium, Annotation of biologically relevant ligands in UniProtKB using ChEBI, Bioinformatics 39 (1) (2022).
[59] K. Moriyama, Y. Kato, D. Hasegawa, Y. Kurimoto, T. Kawaji, T. Nakamura, N. Kuriyama, Y. Shimomura, O. Nishida, Involvement of ionic interactions in cytokine adsorption of polyethyleneimine-coated polyacrylonitrile and polymethyl methacrylate membranes in vitro, J. Artif. Organs 23 (3) (2020) 240–246.

[60] R. Syto, N.J. Murgolo, E.H. Braswell, P. Mui, E. Huang, W.T. Windsor, Structural and biological stability of the human interleukin 10 homodimer, Biochemistry 37 (48) (1998) 16943–16951.
[61] K. Yamada T. Fau - Kato , K. Kato K. Fau - Kawahara , O. Kawahara K. Fau - Nishimura , O. Nishimura , Separation of recombinant human interleukin-2 and methionyl interleukin-2 produced in Escherichia coli, (0006–291X (Print)).
[62] L.L. Bondoc, J.P. Varnerin, J.C.T. Tang, Resolution of recombinant human interleukin 10 from variants by recycling free flow focusing, Anal. Biochem. 246 (2) (1997) 234–238.
[63] T.A. Luger, A. Kock, M. Danner, Characterization of immunoregulatory cytokines produced by epidermal-cells, Scand. J. Immunology 21 (5) (1985) 455–462.
[64] I.E. Adamopoulos, V. Kuchroo, IL-17A and IL-17F in tissue homeostasis, inflammation and regeneration, Nat. Rev. Rheumatol. 19 (9) (2023) 535–536.
[65] M. Yamamoto, Y. Tabata, L. Hong, S. Miyamoto, N. Hashimoto, Y. Ikada, Bone regeneration by transforming growth factor beta 1 released from a biodegradable hydrogel, J. Controlled Release 64 (1-3) (2000) 133–142.
[66] K. Song, I.S. Yoon, N.A. Kim, D.H. Kim, J. Lee, H.J. Lee, S. Lee, S. Choi, M.K. Choi, H.H. Kim, S.H. Jeong, W.S. Son, D.D. Kim, Y.K. Shin, Glycoengineering of interferon-β 1a improves its biophysical and pharmacokinetic properties, (1932–6203 (Electronic)).
[67] C. Przybylski, M. Mokaddem, M. Prull-Janssen, E. Saesen, H. Lortat-Jacob, F. Gonnet, A. Varenne, R. Daniel, On-line capillary isoelectric focusing hyphenated to native electrospray ionization mass spectrometry for the characterization of interferon-gamma and variants, Analyst 140 (2) (2015) 543–550.
[68] S.R. Paul, P. Schendel, The cloning and biological characterization of recombinant human interleukin-11, Int. J. Cell Cloning 10 (3) (1992) 135–143.
[69] I. Striz, E. Brabcova, L. Kolesar, A. Sekerkova, Cytokine networking of innate immunity cells: a potential target of therapy, Clin. Sci. 126 (9) (2014) 593–612.
[70] C. Cayrol, J.-P. Girard, Interleukin-33 (IL-33): A nuclear cytokine from the IL-1 family, Immunol. Rev. 281 (1) (2018) 154–168.
[71] F.Y. Liew, J.-P. Girard, H.R. Turnquist, Interleukin-33 in health and disease, Nat. Rev. Immunol. 16 (11) (2016) 676–689.
[72] L. Zhou, V. Todorovic, Interleukin-36: Structure, Signaling and Function, Adv. Exp. Med Biol. 21 (2021) 191–210.
[73] C.M. Henry, G.P. Sullivan, D.M. Clancy, I.S. Afonina, D. Kulms, S.J. Martin, Neutrophil-derived proteases escalate inflammation through activation of IL-36 family cytokines, Cell Rep. 14 (4) (2016) 708–722.
[74] D. Queen, C. Ediriweera, L. Liu, Function and regulation of IL-36 signaling in inflammatory diseases and cancer development, Front. Cell Dev. Biol. 7 (2019).
[75] F.C. Walker, P.R. Sridhar, M.T. Baldridge, Differential roles of interferons in innate responses to mucosal viral infections, Trends Immunol. 42 (11) (2021) 1009–1023.
[76] L.B. Ivashkiv, IFNγ: signalling, epigenetics and roles in immunity, metabolism, disease and cancer immunotherapy, Nat. Rev. Immunol. 18 (9) (2018) 545–558.
[77] C. Vanhaverbeke, J.-P. Simorre, R. Sadir, P. Gans, H. Lortat-Jacob, NMR characterization of the interaction between the C-terminal domain of interferon-γ and heparin-derived oligosaccharides, Biochem. J. 384 (1) (2004) 93–99.
[78] K. Xu, L. Jin, The role of heparin/heparan sulphate in the IFN-γ-led Arena, Biochimie 170 (2020) 1–9.
[79] H. Lortat-Jacob, F. Baltzer, J.-A. Grimaud, Heparin decreases the blood clearance of interferon-γ and increases its activity by limiting the processing of its carboxyl-terminal sequence, J. Biol. Chem. 271 (27) (1996) 16139–16143.
[80] M.S. Douglas, D.A. Rix, J.H. Dark, D. Talbot, J.A. Kirby, Examination of the mechanism by which heparin antagonizes activation of a model endothelium by interferon-gamma (IFN-γ), Clin. Exp. Immunol. 107 (3) (2003) 578–584.
[81] C. Beadling, M.K. Slifka, Regulation of innate and adaptive immune responses by the related cytokines IL-12, IL-23, and IL-27, Arch. Immunol. Et. Ther. Exp. 54 (1) (2006) 15–24.
[82] W.P. Arend, Balance IL-1 IL-1Ra Dis., Cytokine Growth Factor Rev. 13 (4) (2002) 323–340.
[83] Z. Su, X. Tao, Current understanding of IL-37 in human health and disease, Front. Immunol. 12 (2021) 696605.
[84] L. Xie, Z. Huang, H. Li, X. Liu, S. Zheng, W. Su, IL-38: a new player in inflammatory autoimmune disorders, Biomolecules 9 (8) (2019).
[85] T.L. Sorensen, M. Tani, J. Jensen, V. Pierce, C. Lucchinetti, V.A. Folcik, S.X. Qin, J. Rottman, F. Sellebjerg, R.M. Strieter, J.L. Frederiksen, R.M. Ransohoff, Expression of specific chemokines and chemokine receptors in the central nervous system of multiple sclerosis patients, J. Clin. Investig. 103 (6) (1999) 807–815.
[86] J. Sarmiento, C. Shumate, K. Suetomi, A. Ravindran, L. Villegas, K. Rajarathnam, J. Navarro, Diverging mechanisms of activation of chemokine receptors revealed by novel chemokine agonists, PLoS One 6 (12) (2011).
[87] C.E. Hughes, R.J.B. Nibbs, A guide to chemokines and their receptors, FEBS J. 285 (16) (2018) 2944–2971.
[88] C.L. Salanga, T.M. Handel, Chemokine oligomerization and interactions with receptors and glycosaminoglycans: the role of structural dynamics in function, Exp. Cell Res. 317 (5) (2011) 590–601.
[89] H. Crijns, V. Vanheule, P. Proost, Targeting chemokine—glycosaminoglycan interactions to inhibit inflammation, Front. Immunol. 11 (2020).
[90] R.A. Madan, C. Palena, Behind the IL-8 ball in prostate cancer, Nat. Cancer 2 (8) (2021) 775–776.
[91] G.J. Graham, J. MacKenzie, S. Lowe, M. Tsang, J.A. Weatherbee, A. Issacson, J. Medicherla, F. Fang, P.C. Wilkinson, I.B. Pragnell, Aggregation of the chemokine MIP-1 alpha is a dynamic and reversible phenomenon. Biochemical and biological analyses, J. Biol. Chem. 269 (7) (1994) 4974–4978.
[92] M. Ren, Q. Guo, L. Guo, M. Lenz, F. Qian, R.R. Koenen, H. Xu, A.B. Schilling, C. Weber, R.D. Ye, A.R. Dinner, W.J. Tang, Polymerization of MIP-1 chemokine (CCL3 and CCL4) and clearance of MIP-1 by insulin-degrading enzyme, Embo J. 29 (23) (2010) 3952–3966.
[93] S.C. Chen, B. Mehrad, J.C. Deng, G. Vassileva, D.J. Manfra, D.N. Cook, M. T. Wiekowski, A. Zlotnik, T.J. Standiford, S.A. Lira, Impaired pulmonary host defense in mice lacking expression of the CXC chemokine Lungkine, J. Immunology 166 (5) (2001) 3362–3368.
[94] H.E. Broxmeyer, S.H. Cooper, J. Ropa, CXCL15/Lungkine has suppressive activity on proliferation and expansion of multi-potential, erythroid, granulocyte and macrophage progenitors in S-phase specific manner, Blood Cells Mol. Dis. 91 (2021) 102594.
[95] O. Ohneda, K. Ohneda, H. Nomiyama, Z. Zheng, S.A. Gold, F. Arai, T. Miyamoto, B.E. Taillon, R.A. McIndoe, R.A. Shimkets, D.A. Lewin, T. Suda, L.A. Lasky, WECHE: a novel hematopoietic regulatory factor, Immunity 12 (2) (2000) 141–150.
[96] X. Lu, Structure and function of ligand CX3CL1 and its receptor CX3CR1 in cancer, Curr. Med. Chem. 29 (41) (2022) 6228–6246.
[97] P. Wojdasiewicz, A. Poniatowski, A. Kotela, J. Deszczyński, I. Kotela, D. Szukiewicz, The chemokine CX3CL1 (Fractalkine) and its Receptor CX3CR1: occurrence and potential role in osteoarthritis, Arch. Immunol. Et. Ther. Exp. 62 (5) (2014) 395–403.
[98] S. Apostolakis, D. Spandidos, Chemokines and atherosclerosis: focus on the CX3CL1/CX3CR1 pathway, Acta Pharmacol. Sin. 34 (10) (2013) 1251–1256.
[99] S. Morimura, M. Sugaya, S. Sato, Interaction between CX3CL1 and CX3CR1 regulates vasculitis induced by immune complex deposition, Am. J. Pathol. 182 (5) (2013) 1640–1647.
[100] G. Sciume, A. Soriani, M. Piccoli, L. Frati, A. Santoni, G. Bernardini, CX3CR1/CX3CL1 axis negatively controls glioma cell invasion and is modulated by transforming growth factor-beta1, Neuro-Oncol. 12 (7) (2010) 701–710.
[101] S. Ramazi, J. Zahiri, Posttranslational modifications in proteins: resources, tools and prediction methods, Database (2021) (2021).
[102] V. Vanheule, M. Metzemaekers, R. Janssens, S. Struyf, P. Proost, How post-translational modifications influence the biological activity of chemokines, Cytokine 109 (2018) 29–51.
[103] Inna S. Afonina, C. Müller, Seamus J. Martin, R. Beyaert, Proteolytic processing of interleukin-1 family cytokines: variations on a common theme, Immunity 42 (6) (2015) 991–1004.
[104] H. Bronger, V. Magdolen, P. Goettig, T. Dreyer, Proteolytic chemokine cleavage as a regulator of lymphocytic infiltration in solid tumors, Cancer Metastas Rev. 38 (3) (2019) 417–430.
[105] A.-L. Chamorey, N. Magné, X. Pivot, G. Milano, Impact of glycosylation on the effect of cytokines. A special focus on oncology, Eur. Cytokine Netw. 13 (2) (2002) 154–160.
[106] U. Santhanam, J. Ghrayeb, P.B. Sehgal, L.T. May, Post-translational modifications of human interleukin-6, Arch. Biochem. Biophys. 274 (1) (1989) 161–170.
[107] S. Bohnacker, K. Hildenbrand, I. Aschenbrenner, S.I. Müller, J.E.-v Bieren, M. J. Feige, Influence of glycosylation on IL-12 family cytokine biogenesis and function, Mol. Immunol. 126 (2020) 120–128.
[108] G. Opdenakker, P.M. Rudd, M. Wormald, R.A. Dwek, J. Van Damme, Cells regulate the activities of cytokines by glycosylation, Faseb J. 9 (5) (1995) 453–457.
[109] P. Proost, S. Struyf, J. Van Damme, P. Fiten, E. Ugarte-Berzal, G. Opdenakker, Chemokine isoforms and processing in inflammation and immunity, J. Autoimmun. 85 (2017) 45–57.
[110] H. Denney, M.R. Clench, M.N. Woodroofe, Cleavage of chemokines CCL2 and CXCL10 by matrix metalloproteinases-2 and -9: Implications for chemotaxis, Biochem. Biophys. Res. Commun. 382 (2) (2009) 341–347.
[111] M. Metzemaekers, J. Van Damme, A. Mortier, P. Proost, Regulation of Chemokine Activity – A Focus on the Role of Dipeptidyl Peptidase IV/CD26, Front. Immunol. 7 (2016).
[112] K.M. Sepuru, K. Rajarathnam, Structural basis of chemokine interactions with heparan sulfate, chondroitin sulfate, and dermatan sulfate, J. Biol. Chem. 294 (43) (2019) 15650–15661.
[113] J.H. Cox, R.A. Dean, C.R. Roberts, C.M. Overall, Matrix metalloproteinase processing of CXCL11/I-TAC results in loss of chemoattractant activity and altered glycosaminoglycan binding, J. Biol. Chem. 283 (28) (2008) 19389–19399.
[114] K.Y. Tsang, M.C.H. Cheung, D. Chan, K.S.E. Cheah, The developmental roles of the extracellular matrix: beyond structure to regulation, Cell Tissue Res. 339 (1) (2010) 93–110.
[115] O. Lieleg, R.M. Baumgärtel, A.R. Bausch, Selective filtering of particles by the extracellular matrix: an electrostatic bandpass, Biophys. J. 97 (6) (2009) 1569–1577.
[116] Y.S. Kanwar, A. Linker, M.G. Farquhar, Increased permeability of the glomerular basement membrane to ferritin after removal of glycosaminoglycans (heparan sulfate) by enzyme digestion, J. Cell Biol. 86 (2) (1980) 688–693.
[117] E.M. MunŌz, R.J. Linhardt, Heparin-binding domains in vascular biology, Arterioscler. Thromb. Vasc. Biol. 24 (9) (2004) 1549–1557.
[118] H. Margalit, N. Fischer, S.A. Ben-Sasson, Comparative analysis of structurally defined heparin binding sequences reveals a distinct spatial distribution of basic residues, J. Biol. Chem. 268 (26) (1993) 19228–19231.
[119] J.R. Fromm, R.E. Hileman, E.E. Caldwell, J.M. Weiler, R.J. Linhardt, Pattern and spacing of basic amino acids in heparin binding sites, Arch. Biochem. Biophys. 343 (1) (1997) 92–100.
[120] J.R. Fromm, R.E. Hileman, E.E. Caldwell, J.M. Weiler, R.J. Linhardt, Differences in the interaction of heparin with arginine and lysine and the importance of these

basic amino acids in the binding of heparin to acidic fibroblast growth factor, Arch. Biochem. Biophys. 323 (2) (1995) 279–287.
[121] P.R.B. Joseph, K.V. Sawant, J. Iwahara, R.P. Garofalo, U.R. Desai, K. Rajarathnam, Lysines and Arginines play non-redundant roles in mediating chemokine-glycosaminoglycan interactions, Sci. Rep. 8 (1) (2018) 12289.
[122] S. Salek-Ardakani, J.R. Arrand, D. Shaw, M. Mackett, Heparin and heparan sulfate bind interleukin-10 and modulate its activity, Blood 96 (5) (2000) 1879–1888.
[123] T.A. McCaffrey, D.J. Falcone, B. Du, Transforming growth factor-β1 is a heparin-binding protein: Identification of putative heparin-binding regions and isolation of heparins with varying affinity for TGF-β1, J. Cell. Physiol. 152 (2) (1992) 430–440.
[124] C.A. Jones, K.A. Williams, J.J. Finlay-Jones, P.H. Hart, Interleukin 4 production by human amnion epithelial cells and regulation of its activity by glycosaminoglycan binding1, Biol. Reprod. 52 (4) (1995) 839–847.
[125] L.M. Webb, M.U. Ehrengruber, I. Clark-Lewis, M. Baggiolini, A. Rot, Binding to heparan sulfate or heparin enhances neutrophil responses to interleukin 8, Proc. Natl. Acad. Sci. USA 90 (15) (1993) 7158–7162.
[126] D. Clarke, O. Katoh, R. Gibbs, S. Griffiths, M. Gordon, Interaction of interleukin 7 (IL-7) with glycosaminoglycans and its biological relevance, Cytokine 7 (4) (1995) 325–330.
[127] H. Lortat-Jacob, J.-A. Grimaud, Interferon-γ binds to heparan sulfate by a cluster of amino acids located in the C-terminal part of the molecule, FEBS Lett. 280 (1) (1991) 152–154.
[128] D.F. Boyd, P.G. Thomas, Towards integrating extracellular matrix and immunological pathways, Cytokine 98 (2017) 79–86.
[129] R. Fernandez-Botran, J. Yan, D.E. Justus, Binding of interferon γ by glycosaminoglycans: a strategy for localization and/or inhibition of its activity, Cytokine 11 (5) (1999) 313–325.
[130] D.A. Christian, C.A. Hunter, Particle-mediated delivery of cytokines for immunotherapy, Immunotherapy 4 (4) (2012) 425–441.
[131] R. Swami, A. Shahiwala, Impact of physiochemical properties on pharmacokinetics of protein therapeutics, Eur. J. Drug Metab. Pharmacok. 38 (4) (2013) 231–239.
[132] C.A. Boswell, D.B. Tesar, K. Mukhyala, F.P. Theil, P.J. Fielder, L.A. Khawli, Effects of charge on antibody tissue distribution and pharmacokinetics, Bioconjug Chem. 21 (12) (2010) 2153–2163.
[133] T. Maack, V. Johnson, S.T. Kau, J. Figueiredo, D. Sigulem, Renal filtration, transport, and metabolism of low-molecular-weight proteins: a review, Kidney Int. 16 (3) (1979) 251–270.
[134] V. Bocci, Interleukins: clinical pharmacokinetics and practical implications, Clin. Pharmacokinet. 21 (4) (1991) 274–284.
[135] A. Andres-Hernando, B. Dursun, C. Altmann, N. Ahuja, Z. He, R. Bhargava, C. E. Edelstein, A. Jani, T.S. Hoke, C. Klein, S. Faubel, Cytokine production increases and cytokine clearance decreases in mice with bilateral nephrectomy, Nephrol. Dial. Transpl. 27 (12) (2012) 4339–4347.
[136] R. Newton, J. Uhl, M. Covington, O. Back, The distribution and clearance of radiolabeled human interleukin-1 beta in mice, Lymphokine Res. 7 (3) (1988) 207–216.
[137] J. Klapproth, J. Castell, T. Geiger, T. Andus, P.C. Heinrich, Fate and biological action of human recombinant interleukin 1β in the rat in vivo, Eur. J. Immunol. 19 (8) (1989) 1485–1490.
[138] S. Kudo, K. Mizuno, Y. Hirai, T. Shimizu, Clearance and tissue distribution of recombinant human interleukin 1β in rats, Cancer Res. 50 (18) (1990) 5751–5755.
[139] B. Feinberg, R. Kurzrock, M. Talpaz, M. Blick, S. Saks, J. Gutterman, A phase I trial of intravenously-administered recombinant tumor necrosis factor-alpha in cancer patients, J. Clin. Oncol. 6 (8) (1988) 1328–1334.
[140] B. Ferraiolo, J. Moore, D. Crase, P. Gribling, H. Wilking, R. Baughman, Pharmacokinetics and tissue distribution of recombinant human tumor necrosis factor-alpha in mice, Drug Metab. Dispos. 16 (2) (1988) 270–275.
[141] B. Ferraiolo, J. McCabe, S. Hollenbach, B. Hultgren, R. Pitti, H. Wilking, Pharmacokinetics of recombinant human tumor necrosis factor-alpha in rats, Eff. size Number doses nephrectomy, Drug Metab. Dispos. 17 (4) (1989) 369–372.
[142] K.C. Conlon, E. Lugli, H.C. Welles, S.A. Rosenberg, A.T. Fojo, J.C. Morris, T. A. Fleisher, S.P. Dubois, L.P. Perera, D.M. Stewart, Redistribution, hyperproliferation, activation of natural killer cells and CD8 T cells, and cytokine production during first-in-human clinical trial of recombinant human interleukin-15 in patients with cancer, J. Clin. Oncol. 33 (1) (2015) 74.
[143] J. Mule, J. McIntosh, D. Jablons, S. Rosenberg, Antitumor activity of recombinant interleukin 6 in mice, J. Exp. Med. 171 (3) (1990) 629–636.
[144] J.V. Castell, T. Geiger, V. Gross, T. Andus, E. Walter, T. Hirano, T. Kishimoto, P. C. Heinrich, Plasma clearance, organ distribution and target cells of interleukin-6/hepatocyte-stimulating factor in the rat, Eur. J. Biochem. 177 (2) (1988) 357–361.
[145] A.S. Stern, J. Magram, D.H. Presky, Interleukin-12 an integral cytokine in the immune response, Life Sci. 58 (8) (1996) 639–654.
[146] V. Carreño, S. Zeuzem, U. Hopf, P. Marcellin, W.G.E. Cooksley, J. Fevery, M. Diago, R. Reddy, M. Peters, K. Rittweger, A. Rakhit, M. Pardo, A phase I/II study of recombinant human interleukin-12 in patients with chronic hepatitis B, J. Hepatol. 32 (2) (2000) 317–324.
[147] E.V. Granowitz, R. Porat, J.W. Mier, J.P. Pribble, D.M. Stiles, D.C. Bloedow, M. A. Catalano, S.M. Wolff, C.A. Dinarello, Pharmacokinetics, safety and immunomodulatory effects of human recombinant interleukin-1 receptor antagonist in healthy humans, Cytokine 4 (5) (1992) 353–360.
[148] R. Xie, Y. Zhang, N. Zhao, S. Zhou, X. Wang, W. Han, Y. Yu, X. Zhao, Y. Cui, Pharmacokinetics and safety of recombinant human interleukin-1 receptor antagonist GR007 in healthy Chinese subjects, Eur. J. Drug Metab. Pharmacokinet. 44 (2019) 353–360.
[149] M. Imazio, G. Lazaros, M. Gattorno, M. LeWinter, A. Abbate, A. Brucato, A. Klein, Anti-interleukin-1 agents for pericarditis: a primer for cardiologists, Eur. Heart J. 43 (31) (2022) 2946–2957.
[150] J. Petrasek, S. Bala, T. Csak, D. Lippai, K. Kodys, V. Menashy, M. Barrieau, S.-Y. Min, E.A. Kurt-Jones, G. Szabo, IL-1 receptor antagonist ameliorates inflammasome-dependent alcoholic steatohepatitis in mice, J. Clin. Investig. 122 (10) (2012) 3476–3489.
[151] E. Radwanski, G. Perentesis, S. Jacobs, E. Oden, M. Affrime, S. Symchowicz, N. Zampaglione, Pharmacokinetics of interferon α-2b in healthy volunteers, J. Clin. Pharmacol. 27 (5) (1987) 432–435.
[152] M.T. Lotze, Y. Matory, S. Ettinghausen, A. Rayner, S. Sharrow, C. Seipp, M. Custer, S. Rosenberg, In vivo administration of purified human interleukin 2. II. Half life, immunologic effects, and expansion of peripheral lymphoid cells in vivo with recombinant IL 2, J. Immunol. (Baltim., Md.: 1950) 135 (4) (1985) 2865–2875.
[153] M.W. Konrad, G. Hemstreet, E.M. Hersh, P.W. Mansell, R. Mertelsmann, J. E. Kolitz, E.C. Bradley, Pharmacokinetics of recombinant interleukin 2 in humans, Cancer Res. 50 (7) (1990) 2009–2017.
[154] M.A. Cheever, J.A. Thompson, D.E. Kern, P. Greenberg, Interleukin 2 (IL 2) administered in vivo: influence of IL 2 route and timing on T cell growth, Journal of immunology (Baltimore, Md.: 1950) 134(6) (1985) 3895–3900.
[155] A. Ottolenghi, P. Bolel, R. Sarkar, Y. Greenshpan, M. Iraqi, S. Ghosh, B. Bhattacharya, Z.V. Taylor, K. Kundu, O. Radinsky, Life-extended glycosylated IL-2 promotes Treg induction and suppression of autoimmunity, Sci. Rep. 11 (1) (2021) 7676.
[156] H.M. Alvarez, O.-Y. So, S. Hsieh, N. Shinsky-Bjorde, H. Ma, Y. Song, Y. Pang, M. Marian, E. Escandón, Effects of PEGylation and immune complex formation on the pharmacokinetics and biodistribution of recombinant interleukin 10 in mice, Drug Metab. Dispos. 40 (2) (2012) 360–373.
[157] R.D. Huhn, E. Radwanski, S.M. O'Connell, M.G. Sturgill, L. Clarke, R.P. Cody, M. B. Affrime, D.L. Cutler, Pharmacokinetics and immunomodulatory properties of intravenously administered recombinant human interleukin-10 in healthy volunteers, 1996.
[158] C. Sportès, R.R. Babb, M.C. Krumlauf, F.T. Hakim, S.M. Steinberg, C.K. Chow, M. R. Brown, T.A. Fleisher, P. Noel, I. Maric, Phase I study of recombinant human interleukin-7 administration in subjects with refractory malignancy, Clin. Cancer Res. 16 (2) (2010) 727–735.
[159] T.F. Zioncheck, S.A. Chen, L. Richardson, M. Mora-Worms, C. Lucas, D. Lewis, J. D. Green, J. Mordenti, Pharmacokinetics and tissue distribution of recombinant human transforming growth factor beta 1 after topical and intravenous administration in male rats, Pharm. Res. 11 (1994) 213–220.
[160] L.M. Wakefield, T.S. Winokur, R.S. Hollands, K. Christopherson, A.D. Levinson, M.B. Sporn, Recombinant latent transforming growth factor beta 1 has a longer plasma half-life in rats than active transforming growth factor beta 1, and a different tissue distribution, J. Clin. Investig. 86 (6) (1990) 1976–1984.
[161] P.J. Conlon, S. Tyler, K.H. Grabstein, P. Morrissey, Interleukin-4 (B-cell stimulatory factor-1) augments the in vivo generation of cytotoxic cells in immunosuppressed animals, Biotechnol. Ther. 1 (1) (1989) 31–41.
[162] J. Prendiville, N. Thatcher, M. Lind, R. McIntosh, A. Ghosh, P. Stern, D. Crowther, Recombinant human interleukin-4 (rhu IL-4) administered by the intravenous and subcutaneous routes in patients with advanced cancer—a phase I toxicity study and pharmacokinetic analysis, Eur. J. Cancer 29 (12) (1993) 1700–1707.
[163] V. Bocci, A. Pacini, M. Muscettola, L. Paulesu, G. Pessina, Renal metabolism of rabbit serum interferon, J. Gen. Virol. 55 (2) (1981) 297–304.
[164] M. Blick, S.A. Sherwin, M. Rosenblum, J. Gutterman, Phase I study of recombinant tumor necrosis factor in cancer patients, Cancer Res. 47 (11) (1987) 2986–2989.
[165] M. Hashida, R.I. Mahato, K. Kawabata, T. Miyao, M. Nishikawa, Y. Takakura, Pharmacokinetics and targeted delivery of proteins and genes, J. Control. Release 41 (1) (1996) 91–97.
[166] F. Colotta, P. Allavena, A. Sica, C. Garlanda, A. Mantovani, Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability, Carcinogenesis 30 (7) (2009) 1073–1081.
[167] T. Lan, L. Chen, X. Wei, Inflammatory cytokines in cancer: comprehensive understanding and clinical progress in gene therapy, Cells 10 (1) (2021).
[168] K. Gronke, P.P. Hernández, J. Zimmermann, C.S. Klose, M. Kofoed-Branzk, F. Guendel, M. Witkowski, C. Tizian, L. Amann, F. Schumacher, Interleukin-22 protects intestinal stem cells against genotoxic stress, Nature 566 (7743) (2019) 249–253.
[169] M. Karin, F.R. Greten, NF-κB: linking inflammation and immunity to cancer development and progression, Nat. Rev. Immunol. 5 (10) (2005) 749–759.
[170] X. Long, Y. Ye, L. Zhang, P. Liu, W. Yu, F. Wei, X. Ren, J. Yu, IL-8, a novel messenger to cross-link inflammation and tumor EMT via autocrine and paracrine pathways, Int. J. Oncol. 48 (1) (2016) 5–12.
[171] Y. Yang, Y. Cao, The impact of VEGF on cancer metastasis and systemic disease, Semin. Cancer Biol. 86 (2022) 251–261.
[172] T. Floros, A.A. Tarhini, Anticancer cytokines: biology and clinical effects of interferon-α2, interleukin (IL)-2, IL-15, IL-21, and IL-12, Semin. Oncol. 42 (4) (2015) 539–548.
[173] B. Jiang, Aerobic glycolysis and high level of lactate in cancer metabolism and microenvironment, Genes Dis. 4 (1) (2017) 25–27.
[174] S. Rastogi, S.S. Mishra, M.K. Arora, G. Kaithwas, S. Banerjee, V. Ravichandiran, S. Roy, L. Singh, Lactate acidosis and simultaneous recruitment of TGF-β leads to

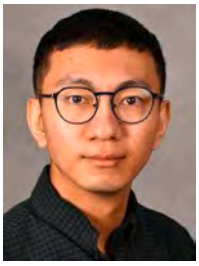
alter plasticity of hypoxic cancer cells in tumor microenvironment, Pharmacol. Ther. 250 (2023) 108519.
[175] K.E. Huntington, A. Louie, L. Zhou, A.A. Seyhan, A.W. Maxwell, W.S. El-Deiry, Colorectal cancer extracellular acidosis decreases immune cell killing and is partially ameliorated by pH-modulating agents that modify tumor cell cytokine profiles, Am. J. Cancer Res. 12 (1) (2022) 138–151.
[176] S. Gaggero, J. Martinez-Fabregas, A. Cozzani, P.K. Fyfe, M. Leprohon, J. Yang, F. E. Thomasen, H. Winkelmann, R. Magnez, A.G. Conti, IL-2 is inactivated by the acidic pH environment of tumors enabling engineering of a pH-selective mutein, Sci. Immunol. 7 (78) (2022) eade5686.
[177] I. Fonda, M. Pernuš, V. Gaberc-Porekar, M. Kenig, A. Štalc, A. Meager, V. Menart, Improvement of potential therapeutic value of tumor necrosis-α (TNF-α) by charge modulation in the tip region, Eur. Cytokine Netw. 16 (1) (2005) 17–26.
[178] H. Lambers, S. Piessens, A. Bloem, H. Pronk, P. Finkel, Natural skin surface pH is on average below 5, which is beneficial for its resident flora, Int. J. Cosmet. Sci. 28 (5) (2006) 359–370.
[179] E.M. Jones, C.A. Cochrane, S.L. Percival, The effect of pH on the extracellular matrix and biofilms, Adv. Wound Care (N. Rochelle) 4 (7) (2015) 431–439.
[180] The Innate Immune System in Acute and Chronic Wounds, Advances in Wound Care 5(2), 2016, pp. 65–78.
[181] S.L. Percival, S. McCarty, J.A. Hunt, E.J. Woods, The effects of pH on wound healing, biofilms, and antimicrobial efficacy, Wound Repair Regen. 22 (2) (2014) 174–186.
[182] R.B. Louisa, N.M. Charne, J.S. Richard, M.B.M. Andrea, S. Geoff, M. William, The pH of wounds during healing and infection: a descriptive literature review, Wound Pract. Res. 25 (2) (2017).
[183] J.A. Kellum, M. Song, J. Li, Science review: extracellular acidosis and the immune response: Clinical and physiologic implications, Crit. Care BioMed. Cent. (2004) 331–336.
[184] O.D. Rotstein, P.E. Nasmith, S. Grinstein, The Bacteroides by-product succinic acid inhibits neutrophil respiratory burst by reducing intracellular pH, Infect. Immun. 55 (4) (1987) 864–870.
[185] T.T. Ward, R.T. Steigbigel, Acidosis of synovial fluid correlates with synovial fluid leukocytosis, Am. J. Med. 64 (6) (1978) 933–936.
[186] H. Peng, J.M. Purkerson, R.S. Freeman, A.L. Schwaderer, G.J. Schwartz, Acidosis induces antimicrobial peptide expression and resistance to uropathogenic E. coli infection in kidney collecting duct cells via HIF-1α, Am. J. Physiol. -Ren. Physiol. 318 (2) (2020) F468–F474.
[187] F. Rippke, E. Berardesca, T.M. Weber, pH and Microbial Infections, Curr. Probl. Dermatol. 54 (2018) 87–94.
[188] I.M. Torres, Y.R. Patankar, B. Berwin, Acidosis exacerbates in vivo IL-1-dependent inflammatory responses and neutrophil recruitment during pulmonaryPseudomonas aeruginosainfection, Am. J. Physiol. -Lung Cell. Mol. Physiol. 314 (2) (2018) L225–L235.
[189] K.L. Gates, H.A. Howell, A. Nair, C.U. Vohwinkel, L.C. Welch, G.J. Beitel, A. R. Hauser, J.I. Sznajder, P.H.S. Sporn, Hypercapnia impairs lung neutrophil function and increases mortality in murine Pseudomonas pneumonia, Am. J. Respir. Cell Mol. Biol. 49 (5) (2013) 821–828.
[190] F. Gül, M.K. Arslantaş, İ. Cinel, A. Kumar, Changing definitions of sepsis, Turk. J. Anaesthesiol. Reanim. 45 (3) (2017), 129-129.
[191] J.E. Gotts, M.A. Matthay, Sepsis: pathophysiology and clinical management, BMJ 353 (2016).
[192] F. Daviaud, D. Grimaldi, A. Dechartres, J. Charpentier, G. Geri, N. Marin, J.-D. Chiche, A. Cariou, J.-P. Mira, F. Pène, Timing and causes of death in septic shock, Ann. Intensive Care 5 (1) (2015).
[193] D.W. Hart, D.L. Chinkes, D.C. Gore, Increased tissue oxygen extraction and acidosis with progressive severity of sepsis, J. Surg. Res. 112 (1) (2003) 49–58.
[194] H.-M. Li, J.H. Jang, J.-S. Jung, J. Shin, C.O. Park, Y.-J. Kim, W.-G. Ahn, J.-S. Nam, C.-W. Hong, J. Lee, Y.-J. Jung, J.-F. Chen, K. Ravid, H.T. Lee, W.-K. Huh, J. H. Kabarowski, D.-K. Song, G2A protects mice against sepsis by modulating kupffer cell activation: cooperativity with adenosine receptor 2b, J. Immunol. 202 (2) (2019) 527–538.
[195] Z. Zhang, C. Zhu, L. Mo, Y. Hong, Effectiveness of sodium bicarbonate infusion on mortality in septic patients with metabolic acidosis, Intensive Care Med. 44 (2018) 1888–1895.
[196] X. Wang, C. Shi, L. Zhang, A. Bodman, D. Guo, L. Wang, W.A. Hall, S. Wilkens, J. Luo, Affinity-controlled protein encapsulation into sub-30 nm telodendrimer nanocarriers by multivalent and synergistic interactions, Biomaterials 101 (2016) 258–271.
[197] L. Wang, C. Shi, X. Wang, D. Guo, T.M. Duncan, J. Luo, Zwitterionic Janus Dendrimer with distinct functional disparity for enhanced protein delivery, Biomaterials 215 (2019) 119233.
[198] X. Ji, X. Yang, C. Shi, D. Guo, X. Wang, J.M. Messina, Q. Meng, N. Urao, R. N. Cooney, J. Luo, Functionalized core–shell nanogel scavenger for immune modulation therapy in sepsis, Adv. Ther. (2022) 2200127.

Jennifer Messina completed her Bachelor of Science in Molecular and Cell Biology and Master of Science in Cell and Developmental Biology at the University of Connecticut in 2019. She is currently an MD/PhD candidate in Dr. Juntao Luo's laboratory in the Department of Pharmacology at SUNY Upstate Medical University. Her PhD research project focuses on nanotechnology development using a novel telodendrimer platform for drug delivery to treat sepsis and chronic critical illness.

Minghao Luo will receive a Bachelor Degree in Clinical Medicine at Harbin Medical University in 2024. Since 2020, Minghao has been involved in research projects on fatty liver and hepatic ischemia-reperfusion in Dr. Jianwei Pan's laboratory in the Department of Pharmacology at Harbin Medical University. He began to join in Dr. Juntao Luo's laboratory remotely at SUNY Upstate Medical University during the COVID-19 Pandemic in 2021. He is dedicated to pursuing a PhD degree in Biomedical Science. He is interested in the translational research in nanomedicine, immune modulation, ischemia-reperfusion, organ transplantation and sepsis.

Md Shanewaz Hossan is currently a Graduate Assistant in Professor Juntao Luo's lab at State University of New York (SUNY) Upstate Medical University and is pursuing his PhD in Biomedical Sciences. His research focuses on Nanotechnology-based Drug Delivery, specifically the development of innovative Telodendrimer nanoparticles for the treatment of sepsis and inflammatory diseases. During his previous professional career, he served as a Research Officer for more than three years at International Centre for Diarrhoeal Disease Research, Bangladesh, where he was engaged in extensive research activities related to antimicrobial resistance, molecular diagnostics, and the development of biomaterials suitable for efficient bioremediation of toxic heavy metals. Mr. Hossan is a decorated alumnus of Jahangirnagar University, having earned his BSC and MS in Microbiology with honors in 2017.

Hadil Gadelrab earned her bachelor's degree in Pharmaceutical Sciences with distinction with honors and her master's degree in Human Genetics from Alexandria University in Egypt. She worked as a hospital pharmacist at Alexandria Main University Hospital for several years. She is currently a Ph.D. candidate in Dr Juntao Luo's Lab in the Pharmacology Department at SUNY Upstate Medical University. Her research interest lies in studying the therapeutic applications of nanomedicine for immune modulation and treatment of ovarian cancer.



Xiguang Yang received his bachelor's degree from Minzu University of China in 2015 and completed his master's degree at the University of Pittsburgh in 2017. After one year in the industry, Xiguang joined the Ph.D. program at SUNY Upstate Medical University in 2018. Xiguang is currently a Ph.D. candidate in the Department of Pharmacology under the guidance of Professor Juntao Luo. Xiguang's research interests center around the development of nanomedicine, molecular tools, and devices, with a foundational commitment to innovating disease treatments. His primary research endeavor involves the development of nanotechnology-based hydrogels to facilitate the healing process of chronic wounds through immune modulation and therapeutics delivery. Xiguang is dedicated to bridging the gap between research and clinical application, aspiring to translate breakthroughs from bench to bedside.

Dr. Joel Wilmore is currently an Assistant Professor in the Department of Microbiology and Immunology at SUNY Upstate Medical University in Syracuse, NY. He obtained a Bachelor of Science in Biotechnology from Rochester Institute of Technology in 2007. He went on to graduate school at SUNY Upstate Medical University where in 2012 he obtained his PhD studying the interface of B cells and malaria in the context of endemic Burkitt's Lymphoma with Dr. Rosemary Rochford. From there, he completed his postdoctoral training with Dr. David Allman at the University of Pennsylvania. During his postdoctoral studies, he became interested in host-microbe interactions and the interplay of commensal microbes and humoral immune responses. He used this new area of research to develop an interest in the protective role of IgA antibodies during sepsis.

Juntao Luo graduated from Nankai University, Tianjin, China with Bsc in Chemistry and Ph.D. degree in Polymer chemistry/physics in 1998 and 2003. He pursued postdoctoral trainings in University of Montreal and University of California Davis Medical Center in bio-/nano-materials, combinatorial chemistry, drug discovery and cancer treatment. In 2011, He joined in the Department of Pharmacology at the State University of New York Upstate Medical University as a tenure-track assistant professor, and he was promoted to Associate Professor and Professor with tenure in 2018 and 2023, respectively. He has developed a novel, well-defined telodendrimer nanoplatform for targeted therapeutic delivery and immune modulation for disease treatments, e.g. cancer, infection and inflammatory diseases. He also holds joint faculty positions in the Department Surgery and the Department Microbiology & Immunology at SUNY Upstate and he is an adjunct faculty in the Department of Chemical and Biomedical Engineering at Syracuse University. He is a founding co-director of Upstate Sepsis Interdisciplinary Research Center. He is also a faculty member of Upstate Cancer Center and Syracuse Bioinspired Institute at Syracuse University.