Future perspective: high-throughput construction of new ultrasensitive cytokine and virion liquid chips for high-throughput screening (HTS) of anti-inflammatory drugs or clinical diagnosis and treatment of inflammatory diseases

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Abstract
Pathogen–host cell interactions play an important role in many human infectious and inflammatory diseases. Several pathogens, including Escherichia coli (E. coli), Mycobacterium tuberculosis (M. tb), and even the recent 2019 novel coronavirus (2019-nCoV), can cause serious breathing and brain disorders, tissue injury and inflammation, leading to high rates of mortality and resulting in great loss to human physical and mental health as well as the global economy. These infectious diseases exploit the microbial and host factors to induce serious inflammatory and immunological symptoms. Thus the development of anti-inflammatory drugs targeting bacterial/viral infection is an urgent need. In previous studies, Yojl-IFNAR2, Yojl-IL10RA, Yojl-NRP1, Yojl-SIGLEC7, and Yojl-MC4R membrane–protein interactions were found to mediate E. coli invasion of the blood–brain barrier (BBB), which activated the downstream anti-inflammatory proteins NACHT, LRR and PYD domains-containing protein 2 (NLRP2), using a proteomic chip conjugated with cell immunofluorescence labeling. However, the studies of pathogen (bacteria/virus)–host cell interactions mediated by membrane protein interactions did not extend their principles to broad biomedical applications such as 2019-nCoV infectious disease therapy. The first part of this feature article presents in-depth analysis of the cross-talk of cellular anti-inflammatory transduction signaling among interferon membrane protein receptor II (IFNAR2), interleukin-10 receptor subunit alpha (IL-10RA), NLRP2 and [Ca²⁺]-dependent phospholipase A2 (PLA2G5), based on experimental results and important published studies, which lays a theoretical foundation for the high-throughput construction of the cytokine and virion solution chip. The paper then moves on to the construction of the novel GPCR recombinant herpes virion chip and virion nano-oscillators for profiling membrane protein functions, which drove the idea of constructing the new recombinant virion and cytokine liquid chips for HTS of leading drugs. Due to the different structural properties of GPCR, IFNAR2, ACE2 and Spike of 2019-nCoV, their ligands will either bind the extracellular domain of IFNAR2/ACE2/Spike or the specific loops of the GPCR on the envelope of the recombinant herpes virions to induce dynamic charge distribution changes that lead to the variable electron transition for detection. Taken together, the combined overview of two of the most innovative and exciting developments in the immunoinflammatory field provides new insight into high-throughput construction of ultrasensitive cytokine and virion liquid chips for HTS of anti-inflammatory drugs or clinical diagnosis and treatment of inflammatory diseases including infectious diseases, acute or chronic inflammation (acute gouty arthritis or rheumatoid arthritis), cardiovascular disease, atheromatosis, diabetes, obesity, tissue injury and tumors. It has significant value in the prevention and treatment of these serious and painful diseases.

Keywords Bacterial/viral infection · Signaling cross-talk · Anti-inflammatory · High-throughput construction · Cytokine/virion liquid
**Abbreviations**

2019-nCoV 2019 Novel coronavirus  
(BBB) Blood–brain barrier  
BSA Bovine serum albumin  
CNF1 Cytotoxic necrotizing factor 1  
DR-18 Decoy-resistant IL-18  
E. coli Escherichia coli  
FACS Fluorescence-activated cell sorting  
G2A G protein-coupled receptor 132  
GlcNAc N-acetylglucosamine  
GPCR G protein-coupled receptor  
GRASP55 Golgi reassembly stacking protein 55  
HBMEC Human brain microvascular endothelial cells  
HPLC-MS High-performance liquid chromatography-mass spectrometry  
HSV-1 Herpes simplex virus-1  
HTS High-throughput screening  
IFNAR2 Interferon alpha/beta receptor subunit 2  
IL Interleukin  
IL-10RA Interleukin-10 receptor subunit alpha  
IL-18 BP IL-18 binding protein  
ISGs Interferon-stimulated genes  
LPS Lipopolysaccharide  
M. tb Mycobacterium tuberculosis  
MS Mass spectrometry  
m/z Mass/charge  
Ni Nickel  
NK Natural killer  
NLRP2 NACHT, LRR and PYD domains-containing protein 2  
OmpA Outer-membrane protein A  
ORF Open reading frame  
OS Oscillatory shear force  
PEG Polyethylene glycol  
PLA2G5 [Ca\(^{2+}\)]-dependent phospholipase A2  
PS Pulsating shear force  
QCM Quartz microbalance  
Rab8a Ras-related protein Rab-8a  
ROS Reactive oxygen species  
Th1 T-helper 1  
VCAM-1 Vascular cell adhesion molecule

**Introduction**

Infectious diseases such as bacterial meningitis induced by *E. coli* are accompanied by serious inflammation and are an important cause of mortality and morbidity in neonates and children. In infants, the rate of mortality from these serious diseases is as high as 10–30% [1, 2], and mortality rates are even higher in deadly viral diseases such as that seen with the COVID-19 pandemic.

*E. coli* invasion of the blood–brain barrier, which exploits microbial and host factors, is the essential step for bacterial meningitis to occur. Although such diseases are usually accompanied by a serious inflammatory response, which has received much attention, the detailed molecular mechanism related to the inflammatory and immunological symptoms has not yet been revealed. In a previous study, we found that YojI-IFNAR2, YojI-IL10RA, YojI-NRP1, YojI-SIGLEC7 and YojI-MC4R membrane–protein interactions mediated the *E. coli*–human brain microvascular endothelial cell (HBMEC) interaction, through the use of human and *E. coli* proteomic chips [HuProtarray (version II)] conjugated cell labeling, fluorescence-activated cell sorting (FACS) flow cytometry separation technology, and confocal immunofluorescence techniques at Johns Hopkins University medical school (Fig. 1, Table 1) [1–5] It was further reported that invasion of HBMEC by *E. coli* causing meningitis also requires outer-membrane protein A (OmpA), invasion of IbeA, IbeB, IbeC and cytotoxic necrotizing factor 1 (CNF1) and rearrangement of the actin cytoskeleton. It has also been shown that surface-exposed loops of OmpA contribute to *E. coli* binding to HBMECs and that OmpA interacts with HBMECs through N-acetylglucosamine (GlcNAc) residues [6]. However, both the IFNAR2 and 1L10RA binding the YojI of *E. coli* activated the downstream NLRP2 protein (NLR protein family), which induced the anti-inflammatory response of HBMEC. It is speculated that it is involved in the type 2 interferon signaling pathway and suppression of autophagy to inhibit the secretion of IL-1β and IL-18 cytokines [3–5, 7, 8, 13]. The newly discovered NLRP2 protein induced by *E. coli* infection and the NLR protein family are important molecular mechanisms for in vivo inflammation and cytokine secretion [1–7].

However, the identification of IFNAR2 and IL10RA as a YojI-interacting protein receptor is a very interesting discovery, and the type II interferon signaling pathway (IFN-α/β) which binds IFNAR2 is the most important antiviral cellular defense mechanism (Table 1) [1]. Both IFNAR2 and IL10RA may inhibit autophagy to increase the cytokine secretion of IL-1β and IL-18, because autophagy can target pro-IL-1β for degradation, and inhibition of autophagy prevents degradation of pro-IL-1β and pro-IL-18, leaving more cytokines freely available in the cytosol for processing and secretion. In addition, inhibition of autophagy leads to activation of the NLRP3 inflammasome and caspase-1-dependent processing of IL-1β and IL-18 cytokines [3–5, 7, 8, 15, 16].

Interleukins (IL) are a group of cytokines secreted by many cell types and can act on a variety of cells by activating and regulating immune cells and mediating the activation, proliferation and differentiation of T and B lymphocytes. They can eliminate infectious pathogens through the induction of secondary mediators and the recruitment of additional immune cells to the site of infection, leading to pyroptosis of the infected cells. Interleukins can also induce the assembly of cytokines, enzymes
that regulate local and systemic inflammation responses. IL-1β and IL-18 can induce IFN-γ in T cells and natural killer (NK) cells, and assemble pro-inflammatory cytokines and cell adhesion molecules to amplify the inflammatory response, which is referred to as cytokine storm. It activates the expression of a large number of interferon-stimulated genes (ISGs) to inhibit viral replication, and performs the tumor-killing function by immune cells and cellular autophagy. It has recently been found that the IL-18 cytokine is a potential immunotherapy target for tumors, which can be directionally engineered by synthetic biology and yeast assembly to become IL-18 mutant (decoy-resistant IL-18, DR-18). It does not bind the natural pseudoreceptor (IL-18BP) that is secreted by many tumor types, which can serve as a tumor marker, but maintains the signaling transduction function to bind the receptor (IL-18Ra/Rβ) to activate NK and CD8 T cells, and replication of immune cells to clear away tumor cells [7, 15].

Table 1 List of the highlighted hits of human membrane proteins identified by YojI on the HuProt chip (*denotes membrane proteins which bind to both YojI and lbeC)

| Gene symbol | Protein size (a.a.) | Function |
|-------------|---------------------|----------|
| *IFNAR2     | 515                 | Interferon alpha/beta receptor 2; associated with IFNAR1 to form the type I interferon receptor. Receptor for interferons alpha and beta. Involved in IFN-mediated association of STAT1, STAT2 and STAT3 activation [1, 2, 5, 6, 14]. |
| *IL10RA     | 578                 | Cell surface receptor for the cytokine IL10 that participates in IL10-mediated anti-inflammatory functions, limiting excessive tissue disruption caused by inflammation. Upon binding to IL10, induces a conformational change in IL10RB, allowing IL10RB to bind IL10 as well. In turn, the heterotetrameric assembly complex, composed of two subunits of IL10RA and IL10RB, activates the kinases JAK1 and TYK2 that are constitutively associated with IL10RA and IL10RB, respectively. These kinases then phosphorylate specific tyrosine residues in the intracellular domain in IL10RA, leading to the recruitment and subsequent phosphorylation of STAT3. Once phosphorylated, STAT3 homodimerizes, translocates to the nucleus and activates the expression of anti-inflammatory genes. In addition, IL10 RA-mediated activation of STAT3 inhibits starvation-induced autophagy [1, 2]. |
As such, IL-1β and IL-18 are important proinflammatory cytokines and inflammatory markers, which can be engineered as new targets of immunotherapy for cancer (Fig. 2). Cytokines play a direct role in the rapid activation of the immune system to respond to emergency signals, and were the first immunotherapeutic drugs approved by the US Food and Drug Administration (FDA) [15].

To date, thirty-eight members are known. IL is the most common and powerful cytokine factor family and includes IL-1β, IL-1α, IL-18, IL-18BP, IL-33, IL-36RA, IL-36β, IL-36γ, IL-37 and IL-38. IL-1β and IL-18 are the main secreted forms of IL-1 and play an important role in infectious (bacteria/virus), inflammatory and autoimmune diseases (Table 2) [6–8, 14–19]. If these cytokines are subjected to ionized fragments, due to their different mass/charge (m/z) ratios, they will run to form distinct semicircle trajectories in an applied magnetic field, which can be differentiated by high-performance liquid chromatography-mass spectrometry (HPLC-MS) (Table 3).

In the downstream events, YojI binding of the IL10RA receptor activates NLRP2 gene expression, as identified by the microarray assay. According to the reported literature, the NLRP2 protein belongs to the NOD-like receptor (NLR) signaling family, along with NLRP1, NLRP3 and NLRP7, but its function is speculated to be contrary to NLRP1,3,7 in a few cell types [8, 16]. The NLR proteins, which lead to NF-kB activation of both transcription of IL-1β and cleavage of pro-1β by caspase-1, can be spontaneously oligomerized to form NLR1,3,7-ASC-caspase-1 inflammasomes, which promote the secretion of IL-1β. We have conducted a pre-experiment that has already knocked down the NLRP2 gene in HBMEC to promote a large quantity of IL-1β secretion (data not shown here). Thus, IFNAR2 and IL10RA inhibit autophagy to increase cytokine secretion, possibly through an NLRP2 protein-mediated inflammasome inhibition mechanism (Fig. 2a) [6–8, 14–19].

In our recent experiment, results confirmed that knockdown of the NLRP2 gene by ShRNA lentivirus combined with low-concentration puromycin (8 μg/mL) treatment induced HBMEC secretion of a large quantity of IL-1β. The NLRP2 protein, in contrast to NLRP3, can inhibit the cellular inflammatory response. Therefore, studies on the ligand–membrane protein interactions associated with an intracellular anti-inflammatory mechanism are very meaningful for the development of recombinant virion and cytokine chips for clinical inflammatory disease detection and therapy (Fig. 2b) [20, 22–26].

Membrane protein virion chips and virion nano-oscillators have been constructed for profiling membrane protein functions and HTS of small-molecule drugs

In previous studies, CD4 and G protein-coupled receptor (GPCR) virion chips and virion nano-oscillators for profiling membrane protein functions were constructed by the author of the present study and Hu et al. in the USA (Fig. 3) [1, 2]. Both single (i.e., CD4) and multi-pass
### Table 2: Protein sequences and functions of the main cytokines IL-1β, IL-18, IL-6 and IL-8

| Gene name | Protein name | Sequence | Function |
|-----------|--------------|----------|----------|
| **IL1β**  | Interleukin-1 beta (mature form: 117-269, 153AA) | APVRLNCTLRDSSQQKSLVMSGPYELKALHLQGQDM EQQVEMFVQGEESNDKIPVALGLKEKLNYLSCV LKDKDPLQESVDPKNYPKKMKERFVFKIEIN NKLEFESAQPWYISTSAENMPFVLGDTGGQ DITDFTMQFVSS | Potent proinflammatory cytokine. Initially discovered as a major endogenous pyrogen, induces prostaglandin synthesis, neutrophil influx and activation, T-cell activation and cytokine production, B cell activation and antibody production, and fibroblast proliferation and collagen production. Promotes Th17 differentiation of T cells. Synergizes with IL12/interleukin-12 to induce IFNγ synthesis from T-helper 1 (Th1) cells [3–6]. |
| **IL18**  | Interleukin-18 (mature form: 37-193aa, 117AA) | YFGK LESKLSVIRNLNDQVLFHQNRPLFEDMT DSDCRDNAPRTIFIISMYKD SQPRGMAVTSVKEKISWLSCENKJISFKEMNPPDNKDTKSIDIFFQORSVPHIDNKMQATESYEGYFLACEKEDELGDRISSMFTQVQED | A proinflammatory cytokine primarily involved in polarized T-helper 1 (Th1) and natural killer (NK) cell immune responses to perform the tumor-killing effect. Upon binding to IL18R1 and IL18RAP, forms a signaling ternary complex which activates NF-kappa-B, triggering synthesis of inflammatory mediators. It can be applied for immunotherapy for cancer. Synergizes with IL12/interleukin-12 to induce IFNγ synthesis from T-helper 1 (Th1) cells and natural killer (NK) cells [18]. |
| **IL6**   | Interleukin-6 (mature form: 30-212aa, 183AA) | VPPGEDSKDVA APHRQPLTSS ERIDKQIRYILDGIALRKE TCNKSNMCCSKEELANELLNPKMAEKGDCFQSGFNEETCLVKITGGLLEFESSLEYQLNRFSSESEEQARAVQVSKVLIQFLOKAKANK_LDAAITDPDTNTATIONKLQAOQWQLQDMTTTHILRSEKFLQSSLARLQRM | Cytokine with a wide variety of biological functions. It is a potent inducer of the acute phase response. Plays an essential role in the final differentiation of B-cells into Ig-secreting cells Involved in lymphocyte and monocyte differentiation. Acts on B and T cells, hepatocytes, hematopoietic progenitor cells and cells of the CNS. Required for the generation of T(H)17 cells. Also acts as a myokine. It is discharged into the bloodstream after muscle contraction and acts to increase the breakdown of fats and to improve insulin resistance. It induces myeloma and plasmacytoma growth and induces nerve cell differentiation [6, 19]. |
| **IL8**   | Interleukin-8 (99AA) | MTSKLAVALLAALISAALEEGAVLPRSAELRCQCIKYTSKFPHKFIKELRVIDSHPCANTEIVKSLSGRELCLDPKENWVQRVVEKFLKRAENS | IL-8 is a chemotactic factor that attracts neutrophils, basophils and T cells, but not monocytes. It is also involved in neutrophil activation. It is released from several cell types in response to an inflammatory stimulus. IL-8(6-77) has a 5–10-fold higher activity on neutrophil activation, IL-8(5-77) has increased activity on neutrophil activation and IL-8(7-77) has a higher affinity to receptors CXCR1 and CXCR2 as compared to IL-8(1-77) [6, 19]. |
| Analytical approaches | Working principle | Applied field | Advantages | Disadvantages |
|-----------------------|-------------------|---------------|------------|---------------|
| HPLC-MS               | Liquid chromatography-mass spectrometry, which uses liquid chromatography as separation system and mass spectrometry as detection system. After the sample is separated from the mobile phase in the mass spectrum, the ion fragments are separated according to the mass number by the mass analyzer of the mass/charge (m/z) spectrum, which is obtained by the detector. | Drug metabolism, cytokines, antibody, enzyme, polypeptide, polysaccharide, polymer analysis and molecular biology | Can analyze multiple cytokines and drugs at the same time. Analyzes biological macromolecular compounds with very large molecular weight; the determination range of protein analysis molecular weight is more than $10^5$ [1, 2, 20, 21]. | |
| FACS                  | After being dyed with specific fluorescent dye, the cells to be tested are put into the sample tube and enter the flow chamber filled with sheath fluid under gas pressure. Under the restriction of sheath fluid, cells are arranged in a single row and ejected from the nozzle of the flow chamber to form a cell column. The latter intersects the incident laser beam vertically, and the cells in the liquid column are excited by the laser to produce fluorescence. A series of optical systems (lenses, apertures, filters, detectors, etc.) in the instrument collect signals of fluorescence, light scattering, light absorption or cell electrical impedance. The computer system collects, stores, displays and analyzes various signals, and generates statistical analysis of various indicators. | Cell surface protein analysis, immune cytochemical typing, exogenous and endogenous gene expression, membrane potential and cell permeability | Can analyze multiple components of cytokines and drugs at the same time, with strong fluorescence signal [1, 2, 20]. | |
| ELISA                 | Sandwich principle of molecular hybridization | High-precision detection of single drug, proteins and cytokines [1, 2] | | It cannot provide molecular structure, and it is difficult to detect and analyze multiple components of drugs at the same time; cannot effectively exclude a false positive, so it has certain limitations. |
| 8%–12% SDS-P-AGE      | Polyacrylamide gel as a support medium for a common electrophoresis technology. It has a molecular sieve effect, which is used to separate cytokines and oligonucleotides. | | In this method, the mobility of cytokines is determined mainly by their molecular weight, not by charge and molecular shape [1]. It cannot determine the specific cytokine. |
(i.e., GPCRs) membrane proteins were exquisitely displayed on the envelopes of herpes simplex virus-1 (HSV-1) with correct orientation and conformation [1, 2, 20]. Single recombinant virion (~185 nm diameter) reacted with polyethylene glycol (PEG) linkers (63 nm in length) to form the virion-PEG complex to be tethered to a gold-coated chip via PEG linkers. With a similar constructive method, a total of 332 (98.5%) GPCR open reading frames (ORFs) of a total of 337 non-odorant GPCRs were subcloned into the UL27 locus (gB) of the herpes virus genome and used to fabricate the VirD-GPCR array for high-content study of their pharmacological activity and preferred drug targets (Fig. 3) [2, 21]. Among the 1400 FDA-approved drugs, 475 (34%) drugs targeting 108 non-odorant GPCRs have been identified. Although there are about 70% known to have the ligand, 30% of the GPCRs are still without an identified ligand [20, 21, 26–31]. Thus, the structure of un-characterized GPCRs and the abovementioned membrane proteins including IFNAR2 and IL10RA or Spike/ACE2 of 2019-nCoV can be further incorporated into the herpes virus envelope or highly expressed extracellular transmembrane domain to induce synthetic antibody to block bacterial/viral infection [1, 2, 9, 27, 29–31]. These recombinant virions are versatile players in the biomedical field. Various methods using a combined proteomics and genomics approach are rapidly being developed; however, utilization of the recombinant membrane protein herpes virions for inflammatory disease diagnosis and treatment has not yet been reported. In order to construct a new recombinant virion solution chip to establish a multi-task platform for clinical biomedical applications, we proposed the recombinant virion solution chip integrated with the properties of an electrochemical sensor for use in clinical diagnosis of inflammatory diseases as well as HTS of anti-inflammatory drugs [20, 23–26]. Because the molecular drug-receptor interactions on these recombinant virions will trigger charge distribution and electron transition to produce electrochemical signaling, IFNAR2 and IL10RA membrane proteins can be applied with detection of electrical signals for HTS of anti-inflammatory molecular drugs (Fig. 3) [1, 6, 14, 20, 21]. Different shear forces such as pulsating (PS) and oscillatory shear force (OS) can also be loaded to gain variable binding kinetics to characterize the drug activity (Fig. 3) [6, 20].
The NLRP2 gene can be knocked down by ShRNA lentivirus for high-throughput construction of a cytokine solution chip

It is reported that the NLRP2 protein can suppress inflammation [3–5, 16, 32]; thus knockdown of the NLRP2 gene in HBMECs may activate the inflammasome and transcription factor NF-kB and autophagosome to promote the expression and secretion of the IL-1β, IL-18, IL-1α, IL-6, TGFβ, IL-8 and IL-33 cytokines.

There are two steps in IL-1β secretion. First, inflammatory signals, such as lipopolysaccharide (LPS), stimulate synthesis and promote accumulation of the cytosol of pro-IL-1β. Additional signals, such as ATP, ion, membrane perturbations or reactive oxygen species (ROS), are also required for inflammasome activation and assembly, leading to caspase-1 activation and processing of pro-IL-1β and eventual secretion of the active cytokines.

Findings showed that autophagy has a positive contribution to the biogenesis and secretion of the pro-inflammatory cytokine IL-1β via an export pathway that depends on Atg5 and inflammasomes, and at least one of the two mammalian Golgi reassembly stacking protein 55 (GRASP55) and Ras-related protein Rab-8a (Rab8a) is required [9–13, 33]. Additionally, PLA2G5 is required for phospholipid hydrolysis and release of IL-1β and IL-18. Knockdown of the NLRP2 gene induces autophagy to promote inflammasome-dependent IL-1β secretion [9–12, 33, 34]. IL-1β lacks any known signal sequence, and the pathways of its secretion in HBMECs are speculated to be mainly through an inflammasome-dependent autophagy mechanism (Table 2, Fig. 2). [9–13, 33, 34].

Based on the results of previous experiments, several unconventional secretion mechanisms are proposed for the secretion of cytokines.

1. Secretion via secretary lysosome. IL-1β may be incorporated into secretary lysosomes that undergo [Ca2+] dependent exocytosis with the release of the mature IL-1β with the help of the PLA2G5.
2. Secretary autophagy: IL-1β-autophagosome may fuse with the plasma membrane to release soluble IL-1β, which is inflammasome-dependent [11–13].
3. Secretion via exosomes: P2RX7 that is activated by ATP leads to the formation of multivesicular bodies (MVBs) containing exocytosis with IL-1β, caspase-1 and other inflammasome components. These MVBs undergo exocytosis with the release of IL-1β after the lysis of the exosome membrane.
4. Secretion via microvesicle shedding. P2RX7 induces immediate shedding of membrane-derived microvesicles containing IL-1β and inflammasome components. These cytokines are then released extracellularly after microvesicle lysis.
5. Release by translocation through permeabilized plasma membrane, which occurs in cells undergoing pyroptosis, due to the sustained activation of the inflammasome.

IL-1β and IL-18 induce the expression of secondary mediators that attract immune cells to the site of the infection. Although IL-1β and IL-18 have a beneficial role in promoting inflammation and eliminating infectious pathogens, mutations that result in constitutive inflammasome activation and the over-production of IL-1β and IL-18 have been linked to auto-inflammatory and autoimmune disorders.

For these reasons, mechanisms that inhibit IL-1β and IL-18 signaling are of great interest to us. Decoy or soluble receptors that sequester IL-1β and IL-1β antagonists and disrupt IL-1 receptor heterodimerization are intrinsic pathways that inhibit IL-1β signaling. Similarly, naturally occurring IL-18 binding protein (IL-18 BP) can prevent IL-18 from binding to its receptor. Based on this bio-analysis of the secretion signaling pathway of IL, three approaches are proposed to increase the extra-cellular release of cytokines and high-throughput construction of the cytokine solution chip for HTS of molecular drugs.

(1) Knockdown of the NLRP2 gene, along with highly expressed PLA2G5 for hydrolysis of the phospholipid monolayer to promote the local release of IL-1β, IL18, etc., in HBMECs.
(2) Knockdown of the NLRP2 gene and activation of probable G protein-coupled receptor 132 (G2A) gene expression to inhibit cell proliferation and promote inflammation to obtain a high quantity of IL-1β and IL-18.
(3) Knockdown of both the NLRP2 gene and cell adhesion molecule gene (VCAM-1) to promote cell suspension and local inflammation to obtain a high titer of IL-1β, IL-18 and other cytokines in HBMECs.

High-throughput construction of new ultrasensitive solid cytokine chips for HTS of anti-inflammatory drugs

Because the binding of small-molecule drugs with cytokines will present different binding kinetics and electrochemical signaling, the cytokine solution chip can employ an electrochemical three-electrode system for detecting the electron transfer between the drugs and cytokines, and the effective lead drugs can be screened [20, 22–26, 34–36]. If the IL-1β and IL-18 are expressed as chimera IL-1β-V5 tag and IL-18-V5 tag, the cytokines can be loaded on the solid glass slide through V5 antibody binding for HTS of molecular drugs based on the ELISA principle. That is, the lead drugs conjugated to the biotin can be screened out by binding the peroxidase-conjugated avidin, which can catalyze the TMB
chromogenic reagent to present a blue color for ultrasensitive identification of the lead drugs (Fig. 4a).

Remarkably, these cytokines can be tethered to the Ni-coated chip via chelation of $(2\times)6\times\text{His tag}$: Ni, if they expressed as chimera IL-1$\beta$-6×His tag $(2\times)$ and IL-18-6×His tag $(2\times)$ (Fig. 4b). By applying an alternating electric field perpendicular to the Ni-coated surface, the variation of the amplitude can characterize the pharmacodynamic activity of the lead drugs (Fig. 4b).

The candidate drugs can also be labeled by CY-5/CY-3 fluorescent dye for HTS of effective drugs by applying a microgravity field [26]. IL-1$\beta$/IL-18 cytokine drug screening has broad application for the diagnosis and treatment of inflammasome-related diseases, including auto-inflammatory disorders, Crohn’s disease, vitiligo, gout, asbestosis, Alzheimer’s disease and even tumors (Fig. 5c).

**High-throughput construction of the liquid and microfluidic cytokine chips for HTS of anti-inflammatory drugs**

If the cytokines were secreted as a cytokine storm, these massive cytokines can be purified individually (Tables 2 and 3). Because lysine is widely present in peptide chains of proteins, the active agent of bovine serum albumin (BSA) can react with these lysines to make them anchor on the BSA-coated glass slide, which exposes the corresponding active groups for HTS of molecular drugs (Fig. 5a).

On the contrary, if the candidate drugs were anchored on the chip surface through the avidin–biotin interaction, the microfluidic chip could be made to let the IL-1$\beta$ cytokine solution flow through the fixed molecular drugs for HTS of candidate drugs, and identify the lead drugs by mass spectrometry (MS) detection, because a high molecular ion peak can be generated according to charge/mass (m/z) of highly ionized cytokines (Table 2, Fig. 5b).

**Recombinant herpes virion liquid electrochemical sensor can be fabricated by high-throughput construction for HTS of anti-inflammatory drugs or clinical diagnosis and treatment of inflammatory diseases**

Different molecular drug targets will generate variable electrochemical signaling for monitoring, due to the different structural properties of single transmembrane (IFNAR2) and multi-transmembrane protein (GPCR)

As just mentioned above, YoJ binds IFNAR2 and triggers the intracellular anti-inflammatory signaling related to the synthesis and secretion of cytokines IL-1$\beta$/IL-18 [1, 2, 7, 8, 15–19]. Thus, the IFNAR2 receptor should retain the anti-inflammatory properties, which can be utilized for molecular drug docking and screening. A model can be used to explain the drug effectiveness: If the drug binds the single transmembrane protein IFNAR2, it mostly binds the extracellular chain antibody for HTS of molecular drugs based on the ELISA principle. If the drug binds the single transmembrane protein IFNAR2, it mostly binds the extracellular chain antibody for HTS of molecular drugs based on the ELISA principle. If the drug binds the single transmembrane protein IFNAR2, it mostly binds the extracellular chain antibody for HTS of molecular drugs based on the ELISA principle.

**Fig. 4 Construction of new ultrasensitive cytokine and virion chips for HTS of anti-inflammatory drugs or clinical diagnosis and treatment of inflammatory diseases.** a Chimera cytokine IL-1$\beta$-V5 tag and IL-18-V5 tag were highly expressed and were loaded on the solid chip through V5 antibody for HTS of molecular drugs based on the ELISA principle. b Chimera protein IL-1$\beta$-6×His tag $(2\times)$ and IL-18-6×His tag $(2\times)$ were expressed and loaded on the Ni-coated solid chip for HTS of molecular drugs of IL-1$\beta$ cytokines.
(27-243aa) of IFNAR2 to exert its pharmacological activity. However, if the drug binds GPCR with seven-transmembrane domains, it can bind different loops of GPCR or the N-terminal of this protein receptor to perform its pharmacological activity (Fig. 6a). The different binding sites between the IFNAR2 and GPCR will generate different electrochemical signaling for monitoring, which is our primary interest.

Fig. 5  High-throughput construction of the cytokine solution chip for HTS of anti-inflammatory drugs. The drug activity can be characterized either by the settlement of micro-fluid, microgravity condition, Cy-5/Cy-3 labeling or ELISA principle.
(IFNAR2/ACE2/Spike)-HSV-1 and (GPCR)-HSV-1 can be rapidly replicated for high-throughput construction of the recombinant virion liquid electrochemical sensor

Due to the similar single-transmembrane structure between Spike of 2019-nCoV, ACE2 of alveolar epithelial cells II (ATII) and IFNAR2 of HBMEC, the (IFNAR2/IL10RA/ACE2/Spike)-HSV-1 and (GPCR)-HSV-1 can be fabricated and rapidly replicated for high-throughput construction of the recombinant virion liquid sensor. Because the detected solution is a non-aqueous solution, and the IR [Deviation caused by I (current) and R (resistance) of the liquid] is much larger, such as blood and virion liquid, a three-electrode system should be applied, comprising a working electrode, an Ag wire as the quasi-reference electrode and a Pt coil as the counter electrode (Fig. 6b). The electrochemical signal will load on the working electrode and counter electrode which are linked to an electrical access loop for detection of the electron transition and electric current passing through [20, 22–25, 37, 38].

The high-throughput construction of a recombinant virion liquid electrochemical sensor is shown in (Fig. 6) [20, 22–25].

Because the single-transmembrane protein IFNAR2 presents a very long extracellular domain (27–243aa), which is similar to CD4 and IFNAR2, Spike (14–1195aa) of 2019-nCoV and ACE2 (18–740aa) of alveolar epithelial cells II, they can be very easily incorporated into the envelope of HSV-1 under the glycoprotein B promoter of the HSV-1 genome to make the IFNAR2/ACE2/Spike-HSV-1 and GPCR recombinant virions (www.uniprot.org) [1, 2, 20, 21, 26]. Therefore, the IFNAR2/ACE2/Spike and GPCR-recombinant herpes virions (IFNAR2/ACE2/Spike-HSV-1 and GPCR-HSV-1) can be created through high-throughput construction to obtain a new virion solution electrochemical sensor for probing drug activity. The applied electrochemical sensor is very sensitive for monitoring electron transition of IFNAR2/IL10RA/ACE2/Spike/GPCR-molecular drug interaction in a small area of the virion solution, which can be compared with the electrical signal of the negative control virions (wild-type virions without IFNAR2/IL10RA/ACE2/Spike or GPCR incorporation) to gain electrical signaling of clinical diagnosis and to identify effective drugs (Fig. 6b) [2, 9, 10, 21, 26–32].

In previous studies, recombinant purified virions were arrayed in a 384-well plate and spotted on FAST slides (Whatman) in a 4 × 4 pattern to fabricate the virion display (VirD) array in order to profile functional membrane proteins using the Cy5-labeled antibody, ligand and lectin to detect immunofluorescence intensity for characterization of their structure and activity (Fig. 1) [2, 20, 21]. Next, virion nano-oscillators were made to allow us to measure mobility and charge changes associated with molecular binding strength on each recombinant virion, characterized by the variation in binding kinetics and amplitude (Fig. 3). These novel analytical characterization approaches and real-time photoelectric measurement techniques provide us a new reference of theoretical analysis for the construction of the recombinant virion liquid electrochemical sensor. It can be applied not only in the detection of infectious diseases (patient blood sample that has been immunized with antibodies of bacteria/virus/2019-nCoV), but also in the detection of diabetes, obesity, tissue injury, acute or chronic inflammation (pancreatitis or rheumatoid arthritis), cardiovascular disease and tumors (Fig. 6b). Subsequently, the screened drugs can be docked to the protein receptors on the virion envelope for designing highly effective anti-inflammatory drugs based on their crystal structure with computer-aided drug design. Because of the flexibility of the VirD platform, we can co-infect human cells with more than one type of VirD-GPCR to produce chimera, heterodimer or multimer VirD-GPCRs to establish the multifunctional virion liquid electrochemical platform for HTS of molecular drugs and the clinical diagnosis and treatment of infectious and inflammatory diseases.

Anti-inflammatory and anti-2019-nCoV drugs can be developed

If we have identified the drugs with strong effectiveness by the virion and cytokine liquid chips, we can obtain the drug-recombinant virion crystal structures to design and modify the drugs based on the membrane protein receptor structure and their electronic binding properties [39–41]. Both the membrane protein receptors (Fig. 6a) and the transcription factor NF-kB and NLRP2 proteins can be designed as drugs for blocking the membrane protein interactions or blocking the protein aggregation and DNA binding properties to suppress inflammation in the human body [36, 42].

Conclusions

We have discovered that the YojI-IFNAR2 and YojI-IL10RA membrane protein interactions mediate anti-inflammatory signaling cross-talk, which is related to the NLRP2 protein inhibition of inflammasome-mediated synthesis and secretion of IL-1β and IL-18. This novel unrevealed anti-inflammatory cellular signaling was applied to investigate the high-throughput construction of liquid cytokine chips and for the development of the single (IFNAR2/IL10RA/ACE2/Spike) and multi-transmembrane protein (GPCR) recombinant herpes virion liquid electrochemical sensor (IFNAR2/IL10RA/ACE2/Spike-HSV-1 and GPCR-
HSV-1) for HTS of anti-inflammatory drugs or clinical diagnosis and treatment of inflammatory diseases. Recently, the RNA coronavirus 2019-nCoV has posed a serious global public health threat, with death rates increasing every day. The infection occurs mainly through the membrane protein interaction of Spike with ACE2. Because the Spike protein is a trimer and ACE2 is a dimer (Fig. 6a), immune response is of tremendous importance for the production of synthetic antibodies to eliminate the 2019-nCoV pathogen. Thus, the molecular and protein drugs, vaccines (the single-transmembrane protein of Spike and ACE2 which removed the transmembrane domain) and antibodies can be developed to block the Spike and ACE2 membrane protein interaction to inhibit the 2019-nCoV infection (Fig. 1) [34–36, 39–41], which can be gained through recombinant herpes virion-immunized mice. Recently, angiotensin-converting enzyme 2 (ACE2) has received much attention. It has been identified as a functional receptor that is related to vasoconstriction, which plays a vital role in the development of hypertension, atherosclerosis and kidney disease, and which requires our further investigation (Fig. 6a). Interestingly, we can knock down the NLRP2 gene to monitor the dynamic conformation variation of ACE2 in response to blood rheology [39]. As a result, studies on the interactions between 2019-nCoV, ACE2 and the host innate immune system, and NLRP2 gene function are critical for infectious, cardiovascular and even tumor disease therapy, and to control serious epidemics. In the future, in addition to microfluidic and electrochemical devices, other advanced physical techniques such as field spectroscopy, quartz microbalance (QCM) and sonic waves can be applied for HTS of drugs. The newly constructed cytokine and virion liquid sensors have significant value for clinical diagnosis and treatment of infectious and inflammatory diseases (Figs. 5 and 6) [27, 36, 42].

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Compliance with ethical standards

No animal experiments were performed in this work. It is in compliance with ethical standards.

Conflict of interest

The authors declare that they have no conflict of interest.

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Future perspective: high-throughput construction of new ultrasensitive cytokine and virion liquid chips for...

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Future perspective: high-throughput construction of new ultrasensitive cytokine and virion liquid chips for...