A nanotrap improves survival in severe sepsis by attenuating hyperinflammation

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Targeting single mediators has failed to reduce the mortality of sepsis. We developed a telodendrimer (TD) nanotrap (NT) to capture various biomolecules via multivalent, hybrid and synergetic interactions. Here, we report that the immobilization of TD-NTs in size-exclusive hydrogel resins simultaneously adsorbs septic molecules, e.g. lipopolysaccharides (LPS), cytokines and damage- or pathogen-associated molecular patterns (DAMPs/PAMPs) from blood with high efficiency (92–99%). Distinct surface charges displayed on the majority of pro-inflammatory cytokines (negative) and anti-inflammatory cytokines (positive) allow for the selective capture via TD NTs with different charge moieties. The efficacy of NT therapies in murine sepsis is both time-dependent and charge-dependent. The combination of the optimized NT therapy with a moderate antibiotic treatment results in a 100% survival in severe septic mice by controlling both infection and hyperinflammation, whereas survival are only 50–60% with the individual therapies. Cytokine analysis, inflammatory gene activation and tissue histopathology strongly support the survival benefits of treatments.
Sepsis is a life-threatening condition caused by an exaggerated inflammatory response of the host to systemic infection. Heterogeneous features of the host and bacterial interactions create a complex, dynamic, and nonlinear disease that is extremely challenging to treat\(^1,2\). The mortality rates in patients with severe sepsis and septic shock remain 30–41\%, despite advanced supportive care\(^3\). The current treatment for sepsis includes antibiotic administration to control the septic foci, fluid resuscitation, and vasopressors as needed to maintain organ perfusion. During sepsis, the release of PAMPs and DAMPs perpetuates systemic inflammation, hemodynamic instability, and organ failure\(^4,5\). Unfortunately, effective therapies to prevent overwhelming inflammation and its sequelae in sepsis are lacking.

Lipopolysaccharide (LPS, a potent PAMP) released from the outer membrane of Gram-negative (GN) bacteria binds to Toll-like receptors (e.g., TLR-4) on immune cells\(^6\), stimulating the production of inflammatory cytokines\(^2\), and triggering severe systemic inflammation in sepsis\(^3,6\). Therapies like anti-TLR-4 antibodies\(^8,10\), and LPS-binding molecules/polymer\(^11-14\), including polymyxin B (PMB, a LPS-binding antibiotic)\(^15,16\), were leveraged to control LPS-related inflammation, but have yet to reduce mortality in patients with sepsis. Therapies developed to neutralize proinflammatory cytokines (e.g., TNF-\(\alpha\)\(^17,19\) and IL-\(\beta\)\(^20,21\)) also failed to improve sepsis mortality in clinical trials\(^22\). We believe that this is due to the biologic variability of inflammatory response in infected patients, and the fact that multiple inflammatory mediators contribute dynamically to systemic inflammation in sepsis. Based on this concept, we believe that therapies attenuating multiple mediators are promising interventions, which could be used in conjunction with antibiotics and supportive care to improve survival of sepsis.

We developed a well-defined linear–dendritic telodendrimer (TD) nanoplatform with the precise and engineerable chemical structures for customized nanocarrier design in drug delivery\(^23,24\). We introduced multiple charges and hydrophobic moieties on the dendritic periphery of TD for efficient protein encapsulation\(^25,26\). The charge and hydrophobic structures are ubiquitously present in inflammatory mediators, including cytokines, LPS, and DAMP/PAMP molecules\(^27\), like cell-free DNA/RNA, extracellular adenosine triphosphate (ATP), and free heme\(^28\). The flexible TD scaffold freely changes its conformation to effectively bind a broad range of inflammatory mediators through a synergistic combination of multivalent electrostatic and hydrophobic interactions. Interestingly, we notice that most proinflammatory cytokines are negatively charged proteins, whereas most anti-inflammatory cytokines are positively charged. This charge disparity may poise the activity regulation by the acidosis during inflammation and sepsis\(^29,30\). In addition, cytokines and DAMPs/PAMPs generally have smaller molecular weights of <30 kDa than abundant serum proteins. Here, we report to rationally synthesize the TD NT in size-exclusive hydrogel resins, e.g., PEGA resin with pore size \(<50\) kDa, to preferentially capture inflammatory mediators with different charges, respectively, for precise and effective immune modulation. Our study demonstrates a 100% survival of severe sepsis in murine models treated with the optimized TD NT in combination with a moderate antibiotic therapy by attenuating both hyperinflammation and infection.

Results

**TD NT for LPS binding.** As illustrated in Fig. 1a, our previous studies have shown TD nanocarrier design for protein encapsulation and delivery based on the synergistic combination of multiple charge and hydrophobic interactions\(^25,26\). Similarly, the negatively charged LPS can be effectively captured by TDs composed of positively charged and hydrophobic moieties as illustrated in Fig. 1b. For nomenclature, TD was named, for example, PEG\(^{5k}(\text{ArgVE})_4\) to refer the PEG (5 kDa)-tethered oligo-glycoline dendron terminated with four arginine (Arg) and four vitamin E (VE). Other acidic or basic amino acids or derivatives, e.g., lysine (Lys), aspartic acid (Asp), and glutamic acid (Glu) or oxalic acid (OA) can be conjugated on TD periphery together with hydrophobic moieties, e.g., heptadecanico acid (C17), vitamin E (VE), and cholester (CHO), respectively, via standard peptide chemistry as shown in Supplementary Fig. 1 following our previous publications\(^25\). TD PEG\(^{5k}(\text{ArgVE})_4\) binds LPS and assemblies into micelles with \(\sim 24\) nm in size, which was smaller than individual TD (30 nm) and LPS (30 nm) nanoparticles (Supplementary Fig. 2). The fluorescent polarization (FP) spectrometry studies indicated the efficient complexation formation between FITC-LPS with TD nanotrips (NTs) than LPS–PMB complexation (Supplementary Fig. 3).

LPS isolated from different GN bacteria, e.g., E. coli and P. aeruginosa, can be efficiently loaded in PEG\(^{5k}(\text{ArgVE})_4\) TD nanoparticles (Fig. 1c), which are stable in the presence of 40-fold excess of PMB (Fig. 1d). In contrast, the PMB–LPS complex with a moderate micromolar-binding affinity\(^32,33\) is less stable and dissociates in electrophoresis (Fig. 1d). As shown in Fig. 1e, the TD–LPS nanocomplex remains stable in the presence of serum protein, which is correlated with the stronger TD–LPS binding than TD–BSA interactions revealed in molecular docking studies (Supplementary Fig. 4). The supplemental data in Supplementary Fig. 5 showed that TD nanocarriers can efficiently trap DAMP molecules, e.g., free DNA and small-molecule-free heme.

**Nanotrap (NT) immobilized on the size-exclusive hydrogel resins.** LPS and most cytokines have relatively small molecular weights (10–30 kDa). Therefore, we conjugated the TD nanotrap on size-exclusive hydrogel resins to selectively capture these inflammatory mediators and exclude the abundant large serum proteins, such as albumin and immunoglobulin (Fig. 2a). We applied standard solid-phase peptide chemistry to synthesize TD stepwise on three hydrogel resins with different pore sizes, e.g., commercial PEGA resin\(^31\) and a synthesized PVA–PEG resin following our previous literature\(^34\) (Supplementary Fig. 6). At the same time, TD was synthesized on a cleavable Rink resin following the same procedure for TD structure confirmation after cleavage. MALDI-TOF MS and NMR analysis confirmed the precise TD synthesis on the hydrogel resins (Supplementary Fig. 7). The nanotrap resins with the arginine and hydrophobic moieties (R) are denoted as RESIN–(ArgR)\(_4\). As expected, the combination of both electrostatic and hydrophobic interactions in TD nanotrap resin is important for effective LPS adsorption: 10–20\% versus 95\% for TD NT resins with individual moieties or combinational interactions (Supplementary Fig. 8A, B). LPS-adsorption capability of PEGA-TD NT resins can be regenerated with sustained adsorption efficiency after four cycles of regeneration, whereas the efficiency of PEGA-PMB was reduced gradually by 50\% (Supplementary Fig. 8C). To test the diffusion kinetics of proteins with different molecular weights, we synthesized two Förster resonance energy transfer (FRET) peptides on PVA–PEG and PEGA resins, e.g., Y(NO\(_2\))\(_2\)diHKSriK(Azb) and Y(NO\(_2\))JayGrGrK(Azb)\(^35\), as substrates for Trypsin (24 kDa) and TNKase (Tencetplase) (45 kDa), respectively. Upon enzymatic cleavage, the fluorescent quencher Y(NO\(_2\))\(_2\) (3-Nitrotyrosine) will be cleaved; Abz(ortho-aminobenzoic acid) moieties on hydrogel resins become fluorescent gradually (Supplementary Fig. 9). As shown in Fig. 2b, smaller trypsin diffused \(\sim 30\)-fold faster than TNKase in PEGA resin, which indicated excellent selectivity toward smaller proteins like cytokines over larger serum proteins. PEGA has a molecular
weight cutoff ~50 kDa, and exhibits a better size-exclusive effect against large proteins than PEG–PVA resin (Supplementary Fig. 9); therefore, it was selected for further studies. PEGA-(ArgVE)₄ resin adsorbs FITC-LPS more efficiently than blank PEGA resin and PEGA-PMB resin, indicated by stronger fluorescence adsorbed on beads (Fig. 2c). Further, confocal images revealed that FITC-LPS and FITC-α-LA (14.2 kDa, PI: 4.5) diffused throughout PEGA-(ArgVE)₄ hydrogel resins without interference by the presence of abundant RB-BSA (1:100 mass ratio), which was restricted to the surface adsorption (Fig. 2d).

**LPS attenuation from biological fluids.** Fetal bovine serum (FBS) and whole blood from healthy human volunteer were doped with FITC-LPS and were incubated with 10% volume of PEGA-(ArgC₁₇)₄ resins and other resins, respectively, e.g., acetylated PEGA blank resin, PEGA-PMB and two commercial...
LPS-binding resins, agarose–PMB resin and cellulose–poly(e)lysine. As shown in Fig. 3a, PEGA-(ArgC17)$_4$ removed FITC-LPS from FBS with ~91% efficiency; PMB-based resins only removed 46–55% of LPS, and polylysine resin was only slightly better than the PEGA control (25% vs. 18%) after 2-h incubation, which was close to the equilibrium as detected after overnight incubation (Supplementary Fig. 10). PEGA-(ArgC17)$_4$ also removed high levels of FITC-LPS from whole blood with an efficiency of ~96% after 2-h incubation (Fig. 3b). LPS elimination from blood by PEGA-PMB (52%), agarose–PMB (64%), and cellulose–polylysine resin (70%) was less effective after 2-h incubation. Accordingly, PEGA-(ArgC17)$_4$ resins showed higher intensity after above-FITC-LPS incubation than all other resins (Supplementary Fig. 11). Unlabeled LPS was added directly or after resin incubations into the culture medium of murine macrophage RAW 264.7 cells. Culture medium was collected after overnight incubation, and TNF-α level was analyzed by ELISA (Fig. 3c). PEGA-TD resins inhibited TNF-α production by >90%, which was significantly better than the PMB-containing PEGA resin (51%) and agarose resin (60%). Polylysine-modified cellulose resin exhibited similar TNF-α attenuation with the control resin by 25%, which was correlated with its poor LPS removal in FBS (Fig. 3a).

Selective protein adsorption. Since charge interactions are critical in protein binding by TD nanocarriers and TD nanotrap resins, we investigated the isoelectric points (PIs) of critical cytokines in the database$^{96}$. Interestingly, we noticed significant charge disparity in counteracting cytokines in both human (Fig. 4a) and mouse (Supplementary Fig. 12): most proinflammatory cytokines (TNF-α, IL-1, IL-6, IL-12, and HMGB-1) have negative charges with PIs ranging between 4.1 and 6.4, while most common anti-inflammatory cytokines (IL-10, TGF-β, IL-4, and IL-11) have positive charges (PIs: 8.2–11.7). We can simply change the charge groups in TD NT to target-specific group of cytokines for potential precise immune modulation. Two model proteins were selected to mimic proinflammatory and anti-inflammatory cytokines for adsorption test, e.g., α-lactalbumin (α-LA, 14.2 kDa, PI: 4.5) and lysozyme (Lyz, 14.4 kDa, PI: 10.7). We synthesized TD nanotrap (NT) moieties on PEGA resins with C17 fatty acid as hydrophobic moieties, which further were conjugated with either positively charged arginine (Arg) or negatively charged oxalic acid, yielding NT$^{+}$ PEGA-(ArgC17)$_4$ and NT$^{-}$ PEGA-(OAC17)$_4$, respectively. We then tested the charge selectivity in protein adsorption as shown in Fig. 4b. As expected, the attractive electrostatic interactions significantly improve protein adsorption in NTs; for example, the negative protein α-LA$^{-}$ was adsorbed much more efficiently in PEGA-(ArgC17)$_4$, NT$^{+}$ than the positively charged lysozyme$^{+}$ (~95% vs. 25% after 30-min incubation); vice versa, NT$^{-}$ adsorbs lysozyme$^{-}$ much more efficiently than trapping α-LA$^{-}$. Interestingly, Arg-containing NT$^{+}$ exhibited much faster adsorption kinetics than the OA-containing NT$^{-}$ with the identical C17 moieties for both α-LA and lysozyme, which may be due to the capability of guanidinium for hydrogen bonding and pi-cation interactions with proteins. Both PEGA-(ArgC17)$_4$ and PEGA-(ArgVE)$_4$ resins with positive charges scavenged FITC-α-LA efficiently (94 and 96% after 2-h incubation, respectively, Fig. 4c), whereas, only 10% of α-LA was trapped physically in PEGA control resin. The PEGA-PMB resin also removed ~74% of negatively charged α-LA from FBS, owing to the positive charge and hydrophobic features of PMB (Fig. 4c).

A protein mixture of α-LA, Lyz, and BSA (1:1:10 in mass ratio) was incubated with PEGA-(ArgC17)$_4$ resin to study the kinetics and selectivity of protein adsorption. α-LA concentration significantly decreased compared with both larger BSA and positively charged Lyz after 30-min incubation, and was almost undetectable after 1-h incubation by MALDI-TOF MS (Fig. 4d). A neutral small protein myoglobin$^{60}$ (Mb, PI: 7.1, 167 kDa) was added to the above protein mixture, then incubated with different charged nanotrap resins, respectively. As shown in Fig. 4e, blank PEGA resin did not alter the relative intensities of proteins compared with the stock mixture. The positively charged PEGA-(ArgC17)$_4$ resin adsorbed α-LA efficiently from the solution, and a noticeable decrease of neutral protein Mb was also observed. In contrast, the negatively charged PEGA-(OAC17)$_4$ resin completely scavenged the positively charged Lyz (PI: 11.6) with α-LA remaining in the solution. Mb was also removed more efficiently by NT$^{-}$ PEGA-(OAC17)$_4$ than NT$^{+}$ PEGA-(ArgC17)$_4$, maybe due to the chelation effects of oxalic acid (OA) with iron–heme complex in Mb. The absorbed α-LA and Lyz were eluted from resins with 8 M urea treatment, then analyzed by MALDI-TOF MS (Fig. 4f). Weak signals were observed in the elution from blank PEGA resin. In contrast, dominant signals of α-LA or Lyz were detected in the elution from the positive and negative nanotrap resins, respectively, confirming the efficient charge selectivity for protein adsorption. The loading capacity of α-LA in PEGA-(ArgC17)$_4$ resin was detected by MALDI-TOF MS analysis to be ~13 μg of α-LA per mg resin using BSA as an internal reference (Supplementary Fig. 13).
Inflammatory modulation in sepsis treatment. Sepsis models induced by cecum ligation and puncture (CLP) mimic the pathogenesis and progression of human sepsis. To test cytokine adsorption effects of NT resins, septic mice were sacrificed 24 h after CLP (Fig. 5a), and plasma was collected for incubation for 2 h with NT(+) resin PEGA-(ArgC17)₄ at 1:1 plasma/resin volume ratio to mimic the duration for clinical hemoperfusion. As shown in Fig. 5b, NT(+) resin incubation significantly reduced the levels of IL-1β and IL-6 from septic plasma with 93.7–98.6% efficiency analyzed by ELISA. Early cytokine TNF-α peaks at 2–4 h in mice post CLP induction, and was detected at relatively low concentration in septic plasma at 24 h post CLP, which was even undetectable after NT(+) resin incubation. At the same time, NT(+) resin also decreased the positively charged IL-10 levels by 70% (Fig. 5b), which may also help to prevent the development of immunosuppression.

Next, we directly applied blank PEGA resin and PEGA-(ArgC17)₄ NT(+) resin to the right side of abdominal cavity immediately after CLP to examine the effects of local immune intervention on mortality (Fig. 5c). As shown in Fig. 5d, septic mortality was 62.5% 48 h post CLP surgery in control group, and the blank PEGA resin did not alter CLP mortality. Surprisingly, the simultaneous peritoneal application of NT(+) resin actually increased CLP mortality rate to 77.8%. This finding confirms the activity of the NT(+) resin to target proinflammatory cytokines, which however, may be harmful if the initiation of the innate immune response was inhibited in the presence of infections. Therefore, we examined the timing of NT resin application on survival in CLP sepsis, which is clinically relevant since sepsis patients generally have significant inflammatory symptoms at their initial clinical appearance.

We applied NT resins with positive or negative charges in the peritoneal cavity in septic mice at different time after CLP (0 h, 3 h, and 8 h) to target the excessive proinflammatory or anti-inflammatory cytokines in CLP slightly increased survival rate to 37.5%. The delayed applications of both types of NT resins at 3 h and 8 h post CLP significantly improved the survival rates to 50–62.5% compared with CLP alone (25% survival). Interestingly, NT treatments at 8 h post CLP were better than 0-h and 3-h treatments, which was, however, not indicative of the later treatment of the better effect, since mice start to die 24 h post CLP. We further analyzed the blood cell counts over 42 days to monitor the status of the survived mice as shown in Fig. 5f. Survived mice in CLP control group had the lowest levels of the total white blood cells (WBCs) on day 2, followed by a dramatic elevation on day 14, indicating the continuous inflammation with hematological instability.
Interestingly, optimal hematological profile was observed in mice treated with NT(+) at 3 h post CLP, WBC returned back to a normal level on day 7, and remained stable thereafter. In contrast, other treatment groups exhibited much more hematological instability. Especially for NT(-) treatment, which may be due to the spread of the infection with the disabled innate immune response by effective resin attenuation.

**Combination therapy in sepsis treatment.** The effective control of both infection and inflammation is critical to prevent multiple organ failure and improve survival in sepsis. Drug resistance is an increasing challenge in the clinic for infection control. The hypoperfusion in sepsis hinders the drug delivery to the infection sites, thereafter, compromising the antibiotic effect in sepsis. To mimic such clinical scenario, we applied a moderate dose of antibiotic imipenem/cilastatin (IMI, 50/50 mg/kg, 50% of its full dose in mice) at 3 h after CLP to partially control infection in CLP mice. At the same time, we apply both NT(+) and NT(-) resins to modulate immune reactions through intra-peritoneal implantation at 3 h post CLP with or without IMI administration, respectively.

The combination of NT(+) and antibiotics (Fig. 6b) yielded a 100% survival before euthanasia on day 42, which was statistically significant compared with all other groups. Mice treated with...
antibiotics alone had an ~50% mortality on day 7, which was lower than CLP-saline group (mortality >80% on day 7). Similar to the previous treatment studies in Fig. 5e, NT resins alone also yielded comparable survival benefit to the antibiotic treatment, and slower mortality incidences were observed mostly between days 2 and 6 post CLP, whereas mortality events in both CLP and antibiotic groups were concentrated at 24–72 h post CLP, likely due to hyperinflammatory reactions. The combination of NT(−) and antibiotics also significantly reduced the acute-phase mortality. However, more animals in this group were euthanized at the later stage upon severe abdominal abscess formation, indicative of prolonged illness. The body temperature (Fig. 6c) and white blood cell count (Fig. 6d) were recorded for the survived mice over time. The reduced hypothermia and fast temperature recovery were observed in the groups treated with NT(+) with or without antibiotics. CLP mice treated with NT(+) and antibiotics remained the most stable white blood cell counts over a long-term observation with all mice survived. In contrast, large amplitudes of hematological dynamics were observed in other groups, especially for NT(−) groups with/without antibiotics, correlating with the severe abscess formation (Fig. 6b).

Reduced inflammation and tissue damages. To improve sepsis treatment, it is important to demonstrate the evidence-based efficacy in severe sepsis. We conducted a separate batch of CLP studies (n = 5) with the identical procedure and treatments as shown in Fig. 6 with a sham laparotomy group included as a comparison. Body temperature of CLP mice reflects the severity

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of sepsis and correlates well with the mortality. Hypothermic mice with body temperature <30 °C were identified from each group (mostly 21–26 °C), and euthanized at 24 h post CLP for pathophysiological comparison. As shown in Fig. 7a, obvious pathological changes and tissue damages were observed in multiple organs in severe septic mice. Significant features of the acute lung injury (ALI) were shown in CLP-saline mice, e.g., hemorrhage, alveolar thickening, alveoli congestion, and interstitial...
edema. Concurrently, significant intracranial edema and contraction bands were observed in cardiomyocytes in CLP-saline mice (Fig. 7a), which is indicative of early cell death (necrosis) uniquely for cardiac myocytes.45 Significant steatosis and vacuolization in the liver were observed in CLP mice, indicating the dysfunction and hepatocellular injury.27 Significant focal vacuolization, epithelial cell flattening, and desquamation were observed with the resultant luminal dilation, indicating the tubular injury in the kidney. As shown in Fig. 7a, normal intestine has long intact villi with abundant goblet cells to maintain the protective mucus layer. In contrast, the CLP mice showed significant villous shortening, villous edema, villous necrosis, and loss of goblet cells in the intestine, which lead to the increased permeability of the epithelium barrier for microbiome dissemination.

It was noticed that the antibiotic IMI treatment alone did not improve histopathology in all organs as examined in Fig. 7a. Instead, enhanced inflammation and organ damages were observed in the IMI group, e.g., increased alveolar thickening and increased hepatocyte vacuolization, which may be due to the enhanced LPS release after bacterial killing.46 NT(+) resin treatment improved the tissue damages to some extent by scavenging inflammatory mediators, for example, the reduced lung injury and less contract bands in cardiac myocytes. Further, the combination of IMI with NT(+) resins synergistically prevented the tissue damages in all vital organs. In contrast, significant tissue damages were still observed in mice treated with the negatively charged NT(-) resin, which were also improved by IMI combination. However, renal tubular injury and ALI remained significant in the NT(-)/IMI group, indicating possible mortality.

As shown in Fig. 7b, heatmap of multiplex cytokine analysis revealed the significant increase of both proinflammatory and anti-inflammatory cytokines in peritoneal fluids in CLP mice in all treatment groups, e.g., TNF-α, IL-1α, IL-1β, IL-6, MCP-1, and IL-10. In plasma, the key inflammatory cytokines, e.g., IL-6 and IL-10, were also significantly elevated, indicating the spread of systemic inflammation. The individual treatments via antibiotic IMI or NT(+) only reduced the inflammation in individual animals. As expected, the combination of IMI/NT(+) significantly attenuates the inflammation both in peritoneal fluids and in blood (Fig. 7b), especially for TNF-α, IL-6, and IL-10 (Fig. 7c). In contrast, NT(-) treatment significantly increased inflammatory cytokines TNF-α, IL-6, and IL-10, which were even higher than CLP-saline control group. It may be explained by the attenuation of the positively charged anti-inflammatory cytokines by NT(-), which unleashes proinflammatory signaling and in turn leads to even higher IL-10 production.

Significant intestinal hyperemia was induced by CLP. The intestine was harvested at 24 h post surgery for cytokine analysis. As shown in Fig. 8a–c, significant increase in key proinflammatory cytokines, e.g., TNF-α, IL-1β, and IL-6, was observed in the intestine in CLP mice compared with the sham group. CLP mice treated with NT(+)/IMI exhibited the lowest levels of intestinal cytokines among treatment groups and close to the sham group, especially for IL-6. HMGB-1 is a proinflammatory cytokine to propagate inflammation reactions through TLR-4 binding. It is also a typical DAMP molecule indicative of cell damage. As shown in Fig. 8d, the plasma levels of HMGB-1 were significantly increased in all CLP groups, indicating the systemic tissue damage. NT(+)/IMI treatment had the lowest level of plasma HMGB-1 among all treatment groups. The similar trend for HMGB-1 was also observed in both intestine and liver (Fig. 8e, f), indicating the reduced organ inflammation and tissue damages by NT(+)/IMI treatment. NF-κB activation is one of the most important pathway for inflammation in both immune system and organs.47 As shown in Fig. 8g, both NF-κB P65 and p-ικB-α levels in the liver of CLP mice treated with NT(+)/IMI were as low as the sham animals, which were significantly upregulated in other groups (Fig. 8h, i), indicating the activation of NF-κB pathway. In summary, the histological and molecular analysis revealed that the synergistic combination of NT(+) and antibiotics attenuated the excessive inflammation and prevented organ damages, which support the survival benefit in severe sepsis.

Discussion

Multiple signals and pathogenic pathways in sepsis are deemed to be targeted simultaneously in order to improve the survival of sepsis. The application of PMB failed in improving sepsis treatment, because of the dual functions of antibiotics and LPS attenuation. Accordingly, PMB-based Toraymyxin® hemoperfusion therapy also failed in the clinical trials for sepsis treatment.48 The DAMPs and PAMPs perpetuate strong host inflammatory reactions, cytokine storm, and organ damages in sepsis. Our TD nanotrap possesses an "octopus-like" flexible dendritic scaffold, which maximizes the conformational entropy in binding with various inflammatory mediators via the ubiquitous and synergistic charge and hydrophobic interactions, e.g., LPS (Fig. 1), cytokines (Fig. 5), and DAMPs/PAMPs (Supplementary Fig. 5). Conventional adsorption resins are made of hydrophobic polymers for nonspecific adsorption of biomolecules, for example, Cytosorb® for multiple cytokine adsorption. However, the spectrum of molecular adsorption in these cartridges is fixed by the chemistry of the resin, which unfortunately was insufficient to improve the survival of sepsis in the clinical trials.49 In contrast, we chose a hydrophilic, inert, and antifoaming PEG-based PEGA resin for immobilization of versatile TD NT, which avoids cell adhesion (Supplementary Fig. 11). We further examined the biocompatibility of PEGA NT resins by intraperitoneal implantation in mice for 6 months. No noticeable acute or chronic toxicity was observed as evidenced by the normal body weight and blood counting and histological analysis (Supplementary Fig. 16).

The management of hyperinflammatory reactions is as important as effective infection control in bacteremia sepsis, which is even critical for viral sepsis, given no effective antiviral drugs. The precise immune modulation is critical for sepsis treatment because of the dynamic and dysregulated immune system in patients. The disparity of the surface charges in proinflammatory and anti-inflammatory cytokines (Fig. 4a) provides us a unique opportunity for the preferential cytokine attenuation. The charge disparity is conserved in human cytokines (Fig. 4a) and murine cytokines (Supplementary Fig. 12), which ensures the translation of the charge-based TD NT immune modulation from preclinical murine models into the clinical efficacy. Our studies indicated that the application of NT resins with different charges at different time after CLP resulted in different survival benefits in sepsis mice (Fig. 5d, e), emphasizing the importance of precise immune intervention.

We acknowledge that some limitations need to be addressed for clinical translation of TD NT approaches for sepsis treatment. First, we applied NT resin suspension directly in the abdominal cavity of CLP mice, which is not applicable for clinical use in sepsis patients, although it was biocompatible for long-term implantation (Supplementary Fig. 16D). Biodegradable and injectable hydrogels can be applied to tether TD nanotabs for local injection or topical application to attenuate pathogenic biomolecules. Alternatively, it is straightforward to pack TD NT resins into a cartridge for abdominal ultrafiltration or hemoperfusion therapy for clinical sepsis treatments. Second, NT(+) resins for hyperinflammation control will not be applicable to...
sepsis patients in the later stage of immunosuppression. In this case, the application of NT(-) adsorption resins might be beneficial to harness the host immunity for infection control and tissue repair. In addition, this approach may not be sufficient to reverse the multiple organ failures, instead to prevent the progression of MOF by attenuating hyperinflammation.

In summary, we developed a versatile TD NT approach for flexible multivalent charge and hydrophobic moieties on TD can be freely engineered for selective and efficient attenuation of a group of inflammatory mediators for sepsis treatment. We have demonstrated that a combination of NT(+) and a moderate IMI antibiotic treatment yielded a 100% survival in CLP mice in repeated studies in CLP septic mice with different sex and ages. The analysis for both cytokines and inflammatory signaling pathways revealed the significantly reduced local, systemic, and remote organ inflammation, which correlated well with the reduced organ failure, and strongly supports the survival benefit by NT(+)/antibiotics treatment. TD NT resins are readily incorporated into the standard clinical care for sepsis treatment, e.g., local immune intervention or hemoperfusion therapy. If tested effective, it can also be applied to treat other critical illness with high risk of cytokine storm, for example, trauma, burn, and severe cardiac surgery.

**Methods**

**Materials.** All chemicals were used as received, unless otherwise specified. Rink Amide-MBHA resin (HCRAm 04-1-1) was ordered from Nankai HECHEM S&T Co., Ltd (Tianjin, China). Amino PEGA resin (Novabiochem®, Darmstadt, Germany) was obtained from EMD Millipore (Billerica, MA). (Fmoc)-Lys(Boc)-OH, (Fmoc)-Lys(Fmoc)-OH, and trifluoroacetic acid (TFA) were obtained from Chem-Impex International, Inc. (Wood Dale, IL). (Fmoc)-Arg(Pbf)-OH was purchased from AnaSpec Inc. (San Jose, CA). N,N-diisopropylcarbodiimide (DIC), N-hydroxysuccinimide (NHS), succinic anhydride, 4-dimethylaminopyridine (DMAP), and N,N-dimethylformamide, anhydrous (DMF, 99.8%) were received from Acros Organics (Belgium, NJ). Polymyxin B Sulfate, Polymyxin B-Agarose (P1411), LPS from Escherichia coli (L4130), and Pseudomonas aeruginosa (L9143) were purchased from Sigma-Aldrich (St. Louis, MO). Polylysine-cellulose resin (Pierce™) was purchased from Thermo Scientific (Rockford, IL). Limulus amebocyte lysate (LAL) endotoxin quantification kit was purchased from Pierce™ (Thermo Scientific™, IL) and performed following the manufacturer’s instructions. ELISA kits were purchased from companies for direct use (e.g., HMGB-1: Cat. #: NB2P-62677 from Novus Biologicals, IL-1β: Cat. #: BMS6002 from Invitrogen, IL-6 Cat. #: BMS603-2 from Invitrogen, and TNF-α: Cat. #: BMS607HS from Invitrogen); IgG (Cat. #: 34580) was purchased from Thermo Fisher Scientific. HRP-conjugated secondary antibody (Cat. #: sc-516102, 1:4,000) was purchased from Santa Cruz biotechnology, Santa Cruz, CA. PVDF membrane (Cat. #: IPVH00010) from Millipore Co., Ltd. and Bio-Rad protein assay (Cat. #: 50000001) from Bio-Rad Laboratories were used. Antibodies for NF-κB (Cat. #: sc-8008, 1:200), P-icb-α (Cat. #: sc-8404, 1:200), and β-actin (Cat. #: sc-47778, 1:500) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

**Instrumental methods.** Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) spectra were collected on a Bruker Autoflex

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**Fig. 8 Reduced tissue damage and organ inflammation in sepsis survivor.** a–c Critical cytokine expression levels in the intestine. d–f HMGB-1 expression levels in the plasma, liver, and intestine as a DAMP indicator for tissue damage. g–i The expression levels of NF-κB and its activation via phosphorylation of κB-α in the liver as an indicator of remote organ inflammation (n = 3, mean ± SEM. Statistical significance was measured by unpaired one-sided Student’s test). Source data are available in the Source Data file.
III system equipped with a Smart beam II laser source and acquired in positive, reflective mode. 1H NMR spectra were recorded on a 600-MHz Bruker AVANCE NMR spectrometer. Transmission electron microscopy (TEM) characterization of nanoparticles was performed on JEOL JEM-1400 operated at 80 kV. Samples were prepared on glow-discharged carbon-coated copper grids (CF3000- CU, 300 mesh, Electron Microscopy Sciences). The hydrodynamic sizes of nanoparticles were acquired by dynamic light-scattering (DLS) measurement using a particle analyzer (Mastersizer2000, Malvern). The scattering images were recorded at a fixed stack mode having sequential x–y sections taken with a z interval at 5 µm.

Solution-phase telodendrimer synthesis. Telodendrimers bearing both guanidine and hydrophobic groups were initiated from methoxy-terminated amino PEG, MeO–PEG–NH2 (Mw: 5 kDa) following a published procedure. N-terminal-protected lysine was used to synthesize the branched scaffold of polylysine dendrons with polyethylene glycol (PEG) coupling reagent and hydrophobic DMF at room temperature. All reagents are in 3 equiv. stoichiometric excess relative to the primary amine in the intermediates tethered on PEG. The completion of reactions was monitored by the chromogenic ninhydrin tests to probe the consumption of primary amine. dark blue indicates the presence of primary amine, yellow color indicates the completion of amine coupling. Fmoc-protecting group was removed by the treatment of 20% 4-methylpyridinium in DMF for 30 min. Pbf-protecting group was deprotected in the presence of TFA/DCM (50/50, v/v) for 2 h. PEGylated intermediates and telodendrimer products were precipitated from the solution after the reaction by the addition of tenfold volume of ice-chilled ethyl and collected by centrifugation at 1590g. Precipitates were rinsed by ice-chilled ethyl three times and dried in vacuum for further reaction. The final telodendrimer was purified by dialysis and lyophilized for further characterization and applications.

Solid-phase synthesis of LPS-binding moieties. Starting from Tentagel (TG), or PEG, or PVA–PEG resin, (Fmoc)-Lys(Fmoc)-OH, and Fmoc-Oligo (ethylene glycol)-COOH linker were coupled sequentially following the standard peptide synthesis procedures (Supplementary Fig. 6). DIC and HOBt were used as catalytic coupling reagents. All reagents were in threefold excess with respect to the amine functional group on resin. After second-generation dendritic oligolysine synthesis, (Fmoc)-Arg(Pbf)-OH was used to introduce the third layer of oligolysine on acid-orthogonally protected amine groups for charge and hydrophobic moiety conjugation. The hydrodynamic sizes of nanoparticles were measured by a Bio-Rad Universal Hood II Imager (Bio-Rad Laboratories, Inc.) under SYBR Green modes or photographed under UV illumination. The gel was imaged by a Bio-Rad Universal Hood II Imager (Bio-Rad Laboratories, Inc.) under SYBR Green modes or photographed under UV illumination.

Fluorescent polarization assays. The fluorescence polarization (FP) was measured on the Multi-Mode Microplate Reader (SpectraMax i3, Molecular Devices) equipped with a 1.0 suture (COATS, ART 230 A) at about 1.3 cm to the distal end, and was perfused into two holes with a 22-gauge needle. One hole is 0.5 cm from the distal end of the catheter and the other is 0.5 mm further in the catheter. The catheter was then gently squeezed to extrude a small amount of fes (about 1 mm³) from the perfusion sites. The recumbent was returned to the peritoneal cavity, and the peritoneum and skin are closed with 5.0 silk sutures. In sham group, mice were operated following the same protocol without CLP procedure. After operation, mice were resuscitated with 1 mL of warmed saline immediately. The animals were returned immediately to a cage with exposure to an infrared heating lamp for 30 min–1 h, until recovery from anesthesia. Mice were provided with free access to food and water in the bottom of the cage. Buprenorphine (0.05 mg/kg, SQA) was injected for postoperative analgesia every 12 h.

Sepsis treatments in CLP mice. Following the above standard CLP procedure, resuscitation, and pain management procedure post operation, CLP mice were randomly assigned in groups (n = 8–10) for the treatments with NTs, antibiotics, and saline according to the specific experimental design: (1) initial treatment studies in Fig. 5d: 150 µL of blank PEGA resin or NT(8)–PEG(Arg4C17), in 150 µL saline control was added into the peritoneum (n = 10, female BALB/c, 8–10 weeks) through surgery wound right after CLP procedure before wound closure. Animals were monitored frequently for 3 days for mortality comparison. (2) Time and charge effects of NTs in Fig. 5e: 150 µL of NT(8)–PEG(Arg4C17) or NT(8)–PEG(OAc17) resins in 1× PBS were injected into the peritoneal cavity of CLP mice (n = 8–10, female BALB/c, 8–10 weeks) using blunt needle through the surgery wound sites at 0 h, 3 h, or 8 h, respectively, after CLP procedure under Fluriso™ inhalation. Then the incision was re-closed with 3 M Vetbond. Mice were monitored frequently for survival studies over 6 weeks, and body weight was monitored over time. About 100 µL of blood was collected from different timepoints of blood cell counting. The measurement of body temperature was taken by a digital infrared thermometer at the abdomen at different times (5 h, 11 h post surgery, daily for the first week, and once per week afterward). (3) Combination therapy in Fig. 6b: 3 h after CLP procedure, mice (n = 8–10, female BALB/c, 8–10 weeks) were divided randomly for i.p. injections with 150 µL of saline, 150 µL of NT(8)–PEG(Arg4C17) or NT(8)–PEG(OAc17) resins with/without i.p. injection of antibiotics imipenem/cilastin (50/50 mg per kg body weight) every 8 h up to 3 days. Mice were monitored frequently for survival studies, and body weight was monitored over time. About 100 µL of blood was collected from the tail vein at different timepoints for blood cell counting, and body temperature was taken by a digital infrared thermometer at the abdomen daily for the first week, and once per week afterward. (4) Following the same procedure, male CLP mice (n = 8–10, female BALB/c, 8–10 weeks) were treated with saline, IMI, NT(8)–PEG(OAc17), and NT(8)–IMI for survival observations for 6 weeks. Body weight, body temperature, and blood counts were monitored over time (Supplementary Fig. 14). (5) Similarly, aged female BALB/c mice (n = 5, 11 months) were used to create CLP sepsis models, and were treated with saline, antibiotics, IMI, or NT(8)–IMI for survival observations for a week (Supplementary Fig. 15).
Cytoxicity analysis and histological examination. Septic mice were induced by the same CLP procedure (n = 5, female BALB/c, 8–10 weeks) and were treated with the identical regimens, the combination therapy in Fig. 6c: saline, antibiotic IMI, NT(+) Conversely, in IMI- group were included with a laparotomy procedure with cecum taken out and put back into the abdomen before wound closure. Mice were sacrificed 24 h post CLP under anesthesia (ketamine: 100 mg per kg, xylazine: 10 mg per kg). IMI. The peritoneal cavity was rinsed with 600 µL of PBS to collect peritoneal lavage for cytokine analysis. Blood was collected from the inferior vena cava. Peritoneal lavage and plasma were stored at −80 °C for resin treatment or cytokine analysis. Cytokines from plasma and peritoneal lavage were measured by ELISA kit. In total, 0.1 mg in 100 µl of protein extraction buffer. Bio-Rad protein was used to determine protein concentration (Bio-Rad Laboratories, 50000001). In total, 0.1 mg in 100 µl of protein extraction buffer. Bio-Rad protein was used to determine protein concentration (Bio-Rad Laboratories, 50000001).

Characterization of inflammatory activation. Small intestine and liver were collected after mice were sacrificed. The mucosa of the small bowel segments was scraped using a microscope slide. Mucosal scrapings and liver were homogenized using tissue grinder in RIPA lysis and extraction buffer. Bio-Rad protein assay was used to determine protein concentration (Bio-Rad Laboratories, 50000001). In total, 0.1 mg in 100 µl of protein lysis or 50 μg of serum (1:100 dilution) was used for ELISA assay. Biomarkers were measured using commercial ELISA kits according to the manufacturer’s instructions. Heart, liver, lung, kidney, spleen, and intestine were harvested. Part of tissue was processed for protein extraction for NF-κB and HMGB-1 analysis via western blot. The rest of tissue was fixed by 10% neutral buffered formalin in OCT cryo-embedding medium for histologic study. In order to analyze pulmonary structure–function relations, right-side lung lobes were fixed by formalin infusion into the cannulated main bronchus and was immersed in a container of formalin for at least 24 h. Then, the fixed lung tissue was embedded in paraffin for sectioning (5 µm) and then stained with hematoxylin and eosin (H&E) for histopathology analysis.

Statistical analysis. In vitro experiments were conducted in triplicate, and data are presented as mean ± SEM. Nonparametric, one-tailed Student’s t test was performed for comparison of two groups. One-way and two-way analyses of variance (ANOVA) with Newman–Keuls post hoc correction (GraphPad Prism 8) were used for multiple-group analyses. The log-rank (Mantel–Cox) test (GraphPad Prism 8) was used to compare the difference in Kaplan–Meier survival plot between different groups. The level of statistical significance was set with P < 0.05.

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Author contributions

C.S., X.W., and L.W. conducted the experiments and analyzed the data. Q.M., G.D., N.C., and M.D. partially performed experiments and/or analyzed the data. G.W. and C.R. assisted in experimental design and paper editing. J.L. designed experiments, interpreted the data, and prepared the paper.

Competing interests

C.S., L.W., and J.L. are inventors on a related patent submitted by State University of New York Upstate Medical University (International Application No. PCT/US2018/0497, published March 14, 2019). The remaining authors declare no competing interests.

Additional information

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