Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Structural Biology

Amyloid-containing biofilms and autoimmunity

Amanda L. Miller[1], Jaime de Anda[2], Gerard C. L. Wong[2] and Çagla Tükel[1]

Abstract

Bacteria are microscopic, single-celled organisms known for their ability to adapt to their environment. In response to stressful environmental conditions or in the presence of a contact surface, they commonly form multicellular aggregates called biofilms. Biofilms form on various abiotic or biotic surfaces through a dynamic stepwise process involving adhesion, growth, and extracellular matrix production. Biofilms develop on tissues as well as on implanted devices during infections, providing the bacteria with a mechanism for survival under harsh conditions including targeting by the immune system and antimicrobial therapy. Like pathogenic bacteria, members of the human microbiota can form biofilms. Biofilms formed by enteric bacteria contribute to several human diseases including autoimmune diseases and cancer. However, until recently the interactions of immune cells with biofilms had been mostly uncharacterized. Here, we will discuss how components of the enteric biofilm produced *in vivo*, specifically amyloid curli and extracellular DNA, could be interacting with the host's immune system causing an unpredicted immune response.

Addresses

[1] Center for Microbiology and Immunology, Lewis Katz School of Medicine, Temple University, Philadelphia, 19140, Pennsylvania, USA
[2] Department of Bioengineering, Department of Chemistry & Biochemistry, California Nano Systems Institute, University of California, Los Angeles, Los Angeles, 90095, California, USA

Corresponding author: Tükel, Çagla (ctukel@temple.edu)
(Tükel Ç.)

Current Opinion in Structural Biology 2022, **75**:102435

This review comes from a themed issue on **Sequences and Topology**

Edited by **Ilana Lauren Brito** and **Martin Kriegel**

For complete overview of the section, please refer the article collection - Sequences and Topology

Available online 18 July 2022

https://doi.org/10.1016/j.sbi.2022.102435

0959-440X/© 2022 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

The human microbiota includes bacteria, fungi, viruses, and archaea that colonize the barrier and mucosal surfaces including skin, mouth, lungs, and gut. The microbial population of each body part is different, shaped by unique environmental condition. The gut microbiota plays a crucial role in immune and metabolic homeostasis. When this homeostasis is disrupted, opportunistic bacteria can flourish leading to disease states including autoimmune diseases [1–3].

Enteric bacteria that belong to the order Enterobacterales within the class of γ-Proteobacteria colonize the healthy gut as part of the normal microbiota, albeit in relatively low abundances (less than 0.1% of the whole microbiota). Enterobacterales are among the most overgrown symbionts in many conditions involving inflammation such as inflammatory bowel disease, colorectal cancer, and celiac disease [4]. Blooms of these otherwise low abundance bacteria may contribute to disease. There is an increased prevalence of adherent-invasive *Escherichia coli* (*E. coli*) in patients suffering from Crohn's disease and ulcerative colitis, two forms of inflammatory bowel disease [5–7]. Furthermore, the potent inflammatory pathogen-associated molecular pattern (PAMPs), lipopolysaccharide (LPS) is thought to promote disease and has been shown to exacerbate intestinal injury induced by non-steroidal anti-inflammatory agents and celiac disease [8]. Bacteria associated with biofilms decorate their extracellular matrices (ECMs) with PAMPs including the amyloid curli [9]. In colorectal cancer, *E. coli* biofilms directly associate with tumors and contribute to tumorigenesis by producing a DNA-damaging toxin called colibactin [10].

The signals that induce biofilm formation inside a host are not known and may be regulated by the inflammatory environment within the gut. The inflamed gut is a unique microenvironment with gradients of essential nutrients and metabolites that favor growth of pathogenic bacteria. Numerous studies have identified the interactions between the invasive planktonic bacteria and the byproducts produced by the host. The signals that induce biofilm formation inside a host are not known and may be regulated by the inflammatory environment within the gut. In this review, we will discuss how the bacterial amyloid–DNA complexes formed within the enteric biofilm ECM induce or

accelerate the onset of autoimmune diseases and trigger disease flares in susceptible individuals.

Biofilms

In a biofilm, a single bacterial species or multiple species are encapsulated in a three-dimensional ECM adhered to a biotic or abiotic surface [11]. Many bacterial species, including both Gram-negative and Gram-positive species, including the commensal microbes inhabiting the human gastrointestinal tract, can produce biofilms [12]. The composition and the structure of the ECM is specific to the bacterial species and the environment in which it is produced. Biofilms may protect members of the commensal microbiota from the harsh conditions of the gut luminal environment and shear forces. Pathogens produce their own biofilms to compete with the commensal microbiota; these biofilms also protect the bacteria from antimicrobial stressors generated by the immune system [13,14].

The main components of the enteric ECM are amyloid curli, cellulose, BapA, and extracellular DNA (eDNA); the ECM accounts for 75–90% of the total biomass of the biofilm [9,15]. Curli amyloid fibers mediate cell–cell attachment, adhesion to surface, environmental persistence and biofilm formation [16,17]. Cellulose and curli production and secretion are co-regulated by a complex regulatory network that involves the protein CsgD [18,19]. The expression of BapA, a large cell-surface protein required for biofilm formation, is also coordinated with the expression of curli and cellulose, through the action of CsgD [20]. CsgD also regulates expression of the O-antigen capsule, which is critical for environmental persistence, but not for multicellular aggregation [21].

Bacterial amyloid curli

Curli, the main proteinaceous component of a biofilm, forms amyloid fibers that are responsible for biofilm resistance to enzymatic degradation and physical stress. In bacteria lacking the ability to produce curli, the three-dimensional ECM structure is disorganized and strength of the biofilm is decreased [22]. The isolation of curli fibers from a biofilm involves multiple rounds of lysozyme, RNase, and DNase treatments and boiling in sodium dodecyl sulfate, which degrade all ECM components except curli [23]. Harsh conditions such as 90% formic acid and hexafluoroisopropanol are required for the breakdown of curli into its monomeric subunit, CsgA [24]. The complexes formed by curli with the other ECM molecules such as cellulose and eDNA contribute to the structure and stability of the biofilm.

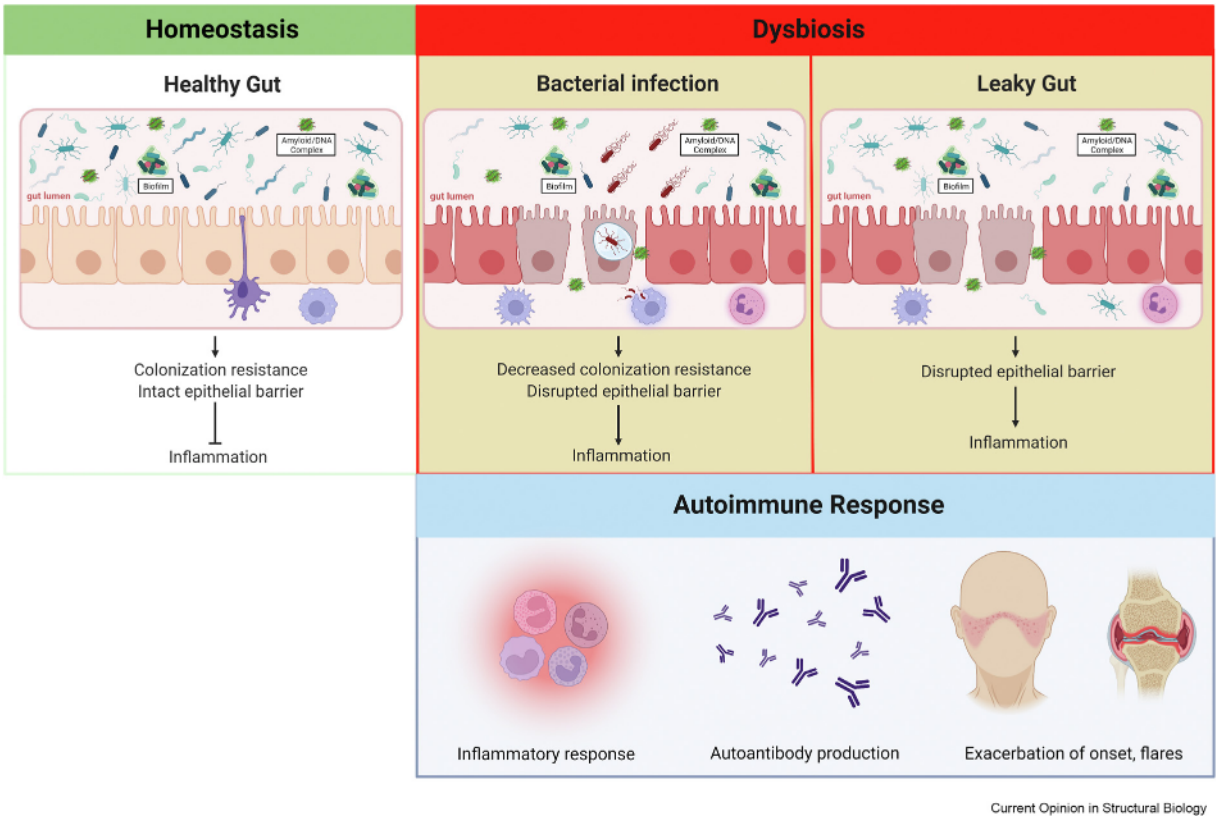
In animal models of *Salmonella enterica* Typhimurium (*S.* Typhimurium) infection and in sepsis patients infected with *E. coli*, antibodies against curli are detected [25,26], and it was recently demonstrated that curli is expressed in the gastrointestinal tract [14], suggesting that biofilms are produced *in vivo*. Curli forms complexes with eDNA that internalize into Toll-like receptor (TLR) 9-containing endosomes of host cells via TLR2 binding. Subsequent recognition of the eDNA in the curli–eDNA complex by TLR9 can lead to the production of type I interferons and anti-double-stranded DNA (dsDNA) autoantibodies (Figure 1) [27,28]. *In vitro* and *in vivo* studies suggest that amyloid curli–eDNA complexes play a role in the pathogenesis of autoimmune diseases including reactive arthritis and systemic lupus erythematosus (SLE) [14,29].

DNA in the biofilm matrix

eDNA is a major constituent of the biofilms of multiple human pathogens [30,31]. eDNA provides structural stability, acts as a sink for antimicrobial peptides, protects resident bacteria from the host immune response, and facilitates the uptake of genetic material between species via horizontal gene transfer [31]. When DNA was first observed in the biofilm matrix of *Pseudomonas aeruginosa (P. aeruginosa)*, it was assumed that the DNA was from lysed cells and that it was not an important component of the biofilm structure. It was soon demonstrated that *P. aeruginosa* produces substantial amounts of DNA through a mechanism independent of cellular lysis, involving the release of small vesicles from the outer membrane [32,33]. Whitchurch and colleagues showed that eDNA is an important functional component of the biofilm ECM. They demonstrated that treatment of *P. aeruginosa* with DNase I prevents biofilm formation and dissolves mature biofilms [31]. Studies using recombinant human DNase I as a prophylactic treatment for cystic fibrosis showed sputum thinning and a decrease in biofilm formation [31].

Autolysis and fratricide are known sources of eDNA in the biofilm, but cell-lysis-independent DNA release by *Bacillus subtilis (B. subtilis)* has been demonstrated [34]. A sequence comparison of DNA released by *B. subtilis* showed that eDNA in the ECM was identical to intracellular DNA, although the two fractions had distinct methylation patterns [34]. Under certain conditions, the classical Watson–Crick-paired, right-handed double helix, a conformation known as B-DNA, can twist in a counterclockwise direction and form a *left-handed* double helix or Z-DNA. Z-DNA is a strong driver of autoimmunity, and antibodies against Z-DNA have been detected in SLE patients [35,36]. Despite these findings, Z-DNA and Z-RNA was thought not to readily occur in nature until a study in 2020 showed that Z-RNA was produced during viral infections and acts as a ligand for the necroptosis-activating host sensor protein ZBP1 [37]. Additionally, Aishwarya et al. recently made the remarkable discovery that the DNA present within *Haemophilus influenzae* (*H. influenzae)*, uropathogenic *E. coli*, and *P. aeruginosa* biofilms is not solely B-DNA but

Figure 1



Amyloid containing biofilms and autoimmunity. Curli–DNA complexes produced by commensal bacteria are recognized by TLR2/TLR1 heterocomplex, which dampens inflammation in healthy intestinal tract. When the epithelial barrier is damaged during invasive infections or by other environmental factors or diseases, curli–DNA complexes dislodged from biofilms activate the TLR2/TLR1 heterocomplex and TLR9 leading to the generation of type I interferons and autoantibodies resulting in initiation or exacerbation of autoimmunity.

also includes substantial amounts of Z-DNA [38]. This notable discovery suggests that biofilms could be a source for of Z-DNA, which leads to generation of autoantibodies in SLE patients and other autoimmune manifestations.

Curli binds tightly to DNA, and both prokaryotic and eukaryotic DNA can be incorporated into the curli fibrils and accelerate the polymerization process [27]. Phenol soluble modulins (PSMs), proteins produced by *Staphylococcus aureus (S. aureus)* also form amyloid fibers, and PSM-eDNA fibers appear to provide structural support in *S. aureus* biofilms [39,40]. Amyloids are not the only proteins that form protein–eDNA complexes within biofilms. The extracellular cell wall protein, LytC, from *Streptococcus pneumoniae,* binds to DNA to form complexes within the biofilm matrix [41]. The nucleoprotein complexes formed in *Myxococcus xanthus* biofilms add mechanical strength and adherence, paralleling the function of curli/eDNA complexes in enteric biofilms [42]. eDNA is also essential to the overall architecture and structural integrity of biofilms formed by non-typeable *H. influenzae* and *Burkholderia cenocepacia*, which have been linked to chronicity, recurrence, and resistance to treatment of multiple respiratory tract diseases. In these biofilms, DNABII proteins bind at the vertices of crossed eDNA strands and act as lynchpins to stabilize the structure of the ECM [43–45]. Inhibition of DNABII binding proteins with antibodies specific to integration host factor (IHF) and/or histone-like protein (HU) induces a collapse of the biofilm and subsequent release of resident bacteria, making them significantly more susceptible to traditional antibiotics. IHF and HU are ubiquitously expressed by eubacteria and have a conserved amino acid sequence homology in the DNA-binding region and a highly conserved three-dimensional conformation that enables the DNABII proteins to bind with high affinity to the eDNA lattice of the biofilm [46].

Amyloid-containing biofilms and autoimmunity

Protein–DNA or protein–RNA complexes of bacterial biofilms are important in the pathogenesis of classical

autoimmune diseases including SLE [47,48]. Our group has shown curli—DNA complexes from either commensal *E. coli* or pathogenic *S.* Typhimurium are recognized by the immune system as a conserved signature, leading to the generation of an autoimmune response characterized by the production of anti-dsDNA and anti-chromatin autoantibodies and type I interferons [27]. Wild-type mice injected with curli—DNA complexes began to develop anti-dsDNA autoantibodies within a week, and the levels of autoantibodies increase over a 6-week period. Interestingly, injections of curli—DNA complexes into TLR2-and TLR9-deficient mice induced very low levels of autoantibodies [28]. A number of studies have now confirmed that curli—eDNA complexes are responsible for the elicitation of immune responses to bacterial biofilms [14,27—29,49—53]. These studies defined a series of events that lead to the severe pro-autoimmune effects of amyloid-expressing bacteria and suggest a mechanism by which the curli acts as a carrier to break immune tolerance to DNA. This is not an enteric specific functionality as this protein/eDNA binding is seen in many other systems.

Both the gut microbiota and infections play roles in the pathogenesis of autoimmune diseases [1,2,54,55]. Autoimmune manifestations are observed in a small percentage of patients after infection with human pathogens such as *Salmonella*, *Yersinia enterocolitica*, *Shigella* spp., *Borrelia burgdorferi*, *Mycobacterium tuberculosis*, *P. aeruginosa*, group A *Streptococci*, and *S. aureus*. Although some of these manifestations are quite puzzling, all of these bacteria produce curli-like amyloids and biofilms [50].

Reactive arthritis, which is triggered by curli-producing enteric pathogens, is relatively well studied compared to the autoimmune sequalae triggered by the other pathogens. Reactive arthritis, characterized by inflammation in the joints, the eyes, and the urethra, occurs in approximately 5% of patients within 1—4 weeks of enteric gastrointestinal infection. Since curli—DNA complexes from enteric biofilms trigger an autoimmune response in mouse models, we investigated whether curli—DNA complexes trigger the disease [14]. Mice orally infected with invasive, curli-producing strains of *S.* Typhimurium or injected intraperitoneally with curli—DNA complexes produced an autoimmune response, but mice orally infected with a non-invasive, curli-producing strain of *S.* Typhimurium or orally administered curli—DNA complexes did not [14]. This indicates that curli produced in the gastrointestinal tract can lead to anti-dsDNA autoantibody production and inflammation in the knee joints of mice when the gut barrier is permeated.

Curli—DNA complexes are also implicated in the pathogenesis of SLE. Factors including lymphopenia, neutropenia, and complement deficiencies likely contribute to the susceptibility of those with SLE to infection, and genetic factors that lead to development of SLE may impair bacterial clearance [56]. SLE patients more frequently experience infections with *S. aureus*, *S.* Typhimurium, *E. coli*, *S. pneumoniae*, and some mycobacterial species [57—59]. Both viral and bacterial infections trigger flares in SLE patients causing flu-like symptoms, fatigue, and muscle and joint pain, and repetitive flare-ups can damage the kidneys and lungs [60]. Infection by curli-producing bacteria like *S.* Typhimurium and *E. coli* can cause disseminated infections in SLE patients leading to bacteremia, septic arthritis, pneumonia, and soft-tissue infections [61—63]. Anti-curli/eDNA antibodies were detected in the plasma of SLE patients; levels were correlated with the presence of asymptomatic persistent bacteriuria and the occurrence of diseases flares [29]. Studies have found increased levels of soluble CD14 and LPS in the blood of SLE patients [64,65], a clear indicator of a damaged mucosal barrier. These findings suggest that curli-expressing bacteria and/or curli—eDNA complexes can pass through the epithelial barrier and are systemically presented to professional immune cells initiating and/or exacerbating autoimmune disease. Additionally, other autoimmune-promoting mechanisms can contribute to SLE pathogenesis by these pathogens; for instance, *S. aureus* via IFN-mediated skin barrier dysfunction [66] or S. Typhimurium via Ro60 orthologs [67,68].

A reduction in the complexity of the microbiota is implicated in the pathogenesis of SLE, rheumatoid arthritis, systemic sclerosis, Sjogren's syndrome, and anti-phospholipid syndrome [1,2,54,55]. In patients with SLE, there is a lower *Firmicutes*/*Bacteroidetes* ratio and higher percentage of *Bacteroidetes* than in healthy controls [54,69]. Analysis of the fecal microbiota showed that the microbiome in patients with SLE was decreased in taxonomic complexity [70]. SLE patients had 5-fold greater representation of *Ruminococcus gnavus* of the Lachnospiraceae family and a reciprocal decrease in species with protective properties. Furthermore, these patients had antibodies against cell-wall lipoglycans of *R. gnavus* in their serum [70]. Another possible pathobiont for SLE in susceptible individuals is the Gram-positive commensal gut bacterium, *Enterococcus gallinarum*. Both healthy individuals and SLE patients were sero-reactive to *E. gallinarum*; however, SLE patients with autoantibodies to ribosomal proteins had higher anti-*E. gallinarum* IgG titers than healthy controls [71]. These higher titers were also significantly associated with the presence of anti-dsDNA, anti-Sm autoantibodies, and antibodies to human RNA [71]. Finally, *E. gallinarum*-specific DNA was recovered from liver biopsies of autoimmune patients suggesting that translocation of this pathobiont into the systemic organs induces autoimmunity [72].

A number of bacterial species express outer-surface proteins with amyloid characteristics. *B. burgdorferi* expresses an amyloid, OspA, that has been shown to induce autoimmunity, specifically Lyme disease; OspA is a molecular mimic of the adhesion molecule LFA-1α, which is a partial agonist of anti-OspA antibodies and exacerbates autoimmune symptoms [73,74]. Although Lyme disease is an infectious disease, many Lyme disease symptoms overlap with those of SLE and patients show positive auto-nuclear antigen (ANA) test results, a diagnostic tool indicative of SLE. These correlations between the two diseases suggest that if untreated, some of the long-term sequelae of Lyme disease can be autoimmune-mediated. A significant antibody and T cell response to OspA develops during prolonged episodes of arthritis [74], suggesting that OspA contributes to autoimmunity. Infection with *M. tuberculosis*, the causative agent of tuberculosis, can also induce autoantibody production and inflammatory arthritis [75–77]. Heat shock proteins from microorganisms can also act as superantigens. Antibodies against HSP65, 70, and 90 have been detected in the sera of patients with SLE [78], and homology between human HSP65 and molecular sequences of *M. tuberculosis* have been identified [79].

As curli or curli-like amyloids are produced by human commensal members [19], leakage of amyloids that are normally confined to the gut could trigger autoimmunity. This idea is consistent with data showing that the presence of curli in the gut is not sufficient to trigger autoimmunity. Mice systemically exposed to purified curli via intraperitoneal injection showed symptoms of autoimmunity but those exposed via oral gavage did not since the complex is unable to escape the gut [14]. Similarly, when mice were implanted subcutaneously with a mesh-associated *S. aureus* biofilm or when PSM3α complexed with DNA is injected systemically into mice, anti-dsDNA autoantibodies were generated in a PSM-dependent manner [80]. We speculate that in conditions where the gut is leaky or with an invasive or chronic systemic infection, bacterial amyloid complexes translocate to the underlying sterile tissues and chronic activation of the immune system with these DNA carriers result in autoimmune reactions.

Relationship between the structure of amyloid–DNA complexes and autoimmunity

Host derived amyloids and antimicrobial peptides (AMPs) were thought to be distinct classes of molecules with drastically different functions: whereas amyloid accumulation in tissues is linked to various disease states, AMPs are best known for their defensive roles in the innate immune system [81]. The lines of demarcation between amyloids and AMPs have blurred in the last 10 years; however, it is now clear that AMPs can be autoantigens. AMPs and amyloids can adopt similar structures and biophysical properties, and both can self-assemble with immune ligands like DNA to amplify immune responses [28,82,83]. Although AMPs and bacterial amyloids have been implicated in the pathogenesis of autoimmune diseases like SLE, psoriasis, rheumatoid arthritis [5,9–13], recent work has shown that many amyloids possess antimicrobial activity, suggesting a potential role in host defense [84]. What's more, given that AMP production increases during bacterial infections, it is possible that 1) AMPs can directly contribute to autoimmunity following infections and 2) AMPs can assemble with amyloids into composite complexes with the same unifying structure and thereby lead to synergistic pro-inflammatory effects. Our preliminary work with hybrid complexes that comprise both host AMPs and "AMP-like" motifs of microbial origin suggests that this latter scenario may be also possible for AMPs and amyloids.

The structures of amyloid and AMP complexes with DNA are critically related to their pro-inflammatory activity. Amyloids can adopt a range of structures. Curli monomers are predicted to adopt a twisted β-sheet secondary structure and assemble into a β-sheet fiber. In contrast, the α-helical PSMs from *Staphylococcus* biofilms are amphiphiles form cross-α fibers composed of two mirrored lattices of helices [40,85]. In both cases, the fibrillation process creates a periodic structure of amino acid motifs along the fiber surface. The exposed hydrophilic interface has the ability to interact with other molecular agents such as eDNA and other amyloids to create macromolecular assemblies.

AMPs can also assemble into protofibril scaffolds that organize double-stranded nucleic acids into nanocrystalline ordered structures with the inter-nucleic acid spacings (a range of values near 35 Å) optimal for multivalent interaction with TLR9 and TLR3, potentially promoting receptor clustering [82,86,87]. This behavior stands in contrast to other cationic molecules, such as cell penetrating peptides like HIV TAT, which bind to DNA and form ordered structures at small inter-DNA spacings that do not activate TLRs. Interestingly, recent work by de Mello et al. demonstrated that a cell penetrating peptide with more hydrophobicity (which made it more similar to AMPs and amyloids) fibrillates in the presence of DNA and can carry DNA into the eukaryotic cells [88].

Interestingly, the general trend in the pro-inflammatory structures of amyloid-DNA and AMP–DNA complexes is evident in pro-inflammatory components responsible for *Clostridiodes difficile*-driven colitis. The *C. difficile* toxin TcdA initiates a marked host innate immune response via TLR9. We recently showed that fragments of TcdA can organize DNA into pro-inflammatory nanocrystalline structures at inter-DNA spacings that activate

TLR9, similar to amyloid–DNA complexes and AMP–DNA complexes [89]. Importantly, even in the protease-rich environment of the gut where only fragments of TcdA exist, the TcdA transduction domain alone can organize DNA into complexes capable of strong multivalent TLR9 activation. Consistent with these results, Di Domizio et al. showed that artificially formed amyloid–DNA complexes administered systematically promote systematic autoimmunity, autoantibody production, and lupus-like syndrome in mice through TLR9 signaling in plasmacytoid dendritic cells [83,90]. In sum, amyloids and AMPs can both organize and chaperone immune ligands into supramolecular structures with optimized geometries that promote multivalent binding to toll-like receptors and thereby amplify immune activation.

Conflict of interest statement

Nothing declared.

Acknowledgments

This work was supported by National Institutes of Health and National Science Foundation. CT is supported by NIH grants AI153325, AI151893, and AI148770. GW, JDA are supported by NSF DMR 1808459 and NIH R37 AI052453. JDA is supported by NSF Graduate Research Fellowship Program DGE-1650604.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sbi.2022.102435.

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. Dehner C, Fine R, Kriegel MA: **The microbiome in systemic autoimmune disease: mechanistic insights from recent studies**. *Curr Opin Rheumatol* 2019, **31**:201–207.

2. Fine RL, Manfredo Vieira S, Gilmore MS, Kriegel MA: **Mechanisms and consequences of gut commensal translocation in chronic diseases**. *Gut Microb* 2020, **11**:217–230.

3. Pereira MS, Redanz S, Kriegel MA: **Skin deep: the role of the microbiota in cutaneous autoimmunity**. *J Invest Dermatol* 2022, **142**:834–840.

4. Amaretti A, Righini L, Candeliere F, Musmeci E, Bonvicini F, Gentilomi GA, Rossi M, Raimondi S: **Antibiotic resistance, virulence factors, phenotyping, and genotyping of non-Escherichia coli Enterobacterales from the gut microbiota of healthy subjects**. *Int J Mol Sci* 2020, **21**.

5. Baumgart M, Dogan B, Rishniw M, Weitzman G, Bosworth B, Yantiss R, Orsi RH, Wiedmann M, McDonough P, Kim SG, Berg D, Schukken Y, Scherl E, Simpson KW: **Culture independent analysis of ileal mucosa reveals a selective increase in invasive Escherichia coli of novel phylogeny relative to depletion of Clostridiales in Crohn's disease involving the ileum**. *ISME J* 2007, **1**:403–418.

6. Darfeuille-Michaud A, Boudeau J, Bulois P, Neut C, Glasser AL, Barnich N, Bringer MA, Swidsinski A, Beaugerie L, Colombel JF: **High prevalence of adherent-invasive Escherichia coli associated with ileal mucosa in Crohn's disease**. *Gastroenterology* 2004, **127**:412–421.

7. Frank DN, St Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR: **Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases**. *Proc Natl Acad Sci U S A* 2007, **104**:13780–13785.

8. Cinova J, De Palma G, Stepankova R, Kofronova O, Kverka M, Sanz Y, Tuckova L: **Role of intestinal bacteria in gliadin-induced changes in intestinal mucosa: study in germ-free rats**. *PLoS One* 2011, **6**, e16169.

9. Tursi SA, Tukel C: **Curli-containing enteric biofilms inside and out: matrix composition, immune recognition, and disease implications**. *Microbiol Mol Biol Rev* 2018, **82**.

10. Allen J, Sears CL: **Impact of the gut microbiome on the genome and epigenome of colon epithelial cells: contributions to colorectal cancer development**. *Genome Med* 2019, **11**:11.

11. Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scott HM: **Microbial biofilms**. *Annu Rev Microbiol* 1995, **49**:711–745.

12. Macfarlane S, Bahrami B, Macfarlane GT: **Mucosal biofilm communities in the human intestinal tract**. *Adv Appl Microbiol* 2011, **75**:111–143.

13. MacKenzie KD, Palmer MB, Koster WL, White AP: **Examining the link between biofilm formation and the ability of pathogenic Salmonella strains to colonize multiple host species**. *Front Vet Sci* 2017, **4**:138.

14. ** Miller AL, Pasternak JA, Medeiros NJ, Nicastro LK, Tursi SA, Hansen EG, Krochak R, Sokaribo AS, MacKenzie KD, Palmer ME, Herman DJ, Watson NL, Zhang Y, Wilson HL, Wilson RP, White AP, Tukel C: **In vivo synthesis of bacterial amyloid curli contributes to joint inflammation during S. Typhimurium infection**. *PLoS Pathog* 2020, **16**, e1008591.
Bacterial amyloid curli is expressed and synthesized in the murine gastrointestinal tract. Leakage of curli from the gut leads to autoimmunity and joint inflammation.

15. Hufnagel DA, Tukel C, Chapman MR: **Disease to dirt: the biology of microbial amyloids**. *PLoS Pathog* 2013, **9**, e1003740.

16. Hammar M, Arnqvist A, Bian Z, Olsen A, Normark S: **Expression of two csg operons is required for production of fibronectin- and Congo red-binding curli polymers in Escherichia coli K-12**. *Mol Microbiol* 1995, **18**:661–670.

17. Romling U, Bian Z, Hammar M, Sierralta WD, Normark S: **Curli fibers are highly conserved between Salmonella typhimurium and Escherichia coli with respect to operon structure and regulation**. *J Bacteriol* 1998, **180**:722–731.

18. White AP, Gibson DL, Kim W, Kay WW, Surette MG: **Thin aggregative fimbriae and cellulose enhance long-term survival and persistence of Salmonella**. *J Bacteriol* 2006, **188**:3219–3227.

19. Zogaj X, Bokranz W, Nimtz M, Romling U: **Production of cellulose and curli fimbriae by members of the family Enterobacteriaceae isolated from the human gastrointestinal tract**. *Infect Immun* 2003, **71**:4151–4158.

20. Latasa C, Roux A, Toledo-Arana A, Ghigo JM, Gamazo C, Penades JR, Lasa I: **BapA, a large secreted protein required for biofilm formation and host colonization of Salmonella enterica serovar Enteritidis**. *Mol Microbiol* 2005, **58**:1322–1339.

21. Gibson DL, White AP, Snyder SD, Martin S, Heiss C, Azadi P, Surette M, Kay WW: **Salmonella produces an O-antigen capsule regulated by AgfD and important for environmental persistence**. *J Bacteriol* 2006, **188**:7722–7730.

22. Hung C, Zhou Y, Pinkner JS, Dodson KW, Crowley JR, Heuser J, Chapman MR, Hadjifrangiskou M, Henderson JP, Hultgren SJ: **Escherichia coli biofilms have an organized and complex extracellular matrix structure**. *mBio* 2014, **4**:e00645. 13.

23. Collinson SK, Parker JM, Hodges RS, Kay WW: **Structural predictions of AgfA, the insoluble fimbrial subunit of Salmonella thin aggregative fimbriae**. *J Mol Biol* 1999, **290**:741–756.

24. Zhou Y, Smith DR, Hufnagel DA, Chapman MR: **Experimental manipulation of the microbial functional amyloid called curli**. *Methods Mol Biol* 2013, **966**:53–75.

25. Bian Z, Brauner A, Li Y, Normark S: **Expression of and cytokine activation by Escherichia coli curli fibers in human sepsis**. *J Infect Dis* 2000, **181**:602–612.

26. Humphries A, Deridder S, Baumler AJ: **Salmonella enterica serotype Typhimurium fimbrial proteins serve as antigens during infection of mice**. *Infect Immun* 2005, **73**:5329–5338.

27. Gallo PM, Rapsinski GJ, Wilson RP, Oppong GO, Sriram U, Goulian M, Buttaro B, Caricchio R, Gallucci S, Tukel C: **Amyloid-DNA composites of bacterial biofilms stimulate autoimmunity**. *Immunity* 2015, **42**:1171–1184.

28. Tursi SA, Lee EY, Medeiros NJ, Lee MH, Nicastro LK, Buttaro B, Gallucci S, Wilson RP, Wong GCL, Tukel C: **Bacterial amyloid curli acts as a carrier for DNA to elicit an autoimmune response via TLR2 and TLR9**. *PLoS Pathog* 2017, **13**, e1006315.

29. * Pachucki RJ, Corradetti C, Kohler L, Ghadiali J, Gallo PM, Nicastro L, Tursi SA, Gallucci S, Tukel C, Caricchio R: **Persistent bacteriuria and antibodies recognizing curli/eDNA complexes from Escherichia coli are linked to flares in systemic lupus erythematosus**. *Arthritis Rheumatol* 2020, **72**:1872–1881.
Anti-curli/eDNA antibodies were detected in the plasma of SLE patients and healthy controls, and their levels correlated with the presence of asymptomatic persistent bacteriuria and occurrence of disease flares in lupus patients. Study suggest that UTIs and persistent bacteriuria incolvin curli producng bacteria are environmental triggers of lupus and its flares.

30. Hall-Stoodley L, Nistico L, Sambanthamoorthy K, Dice B, Nguyen D, Mershon WJ, Johnson C, Hu FZ, Stoodley P, Ehrlich GD, Post JC: **Characterization of biofilm matrix, degradation by DNase treatment and evidence of capsule downregulation in Streptococcus pneumoniae clinical isolates**. *BMC Microbiol* 2008, **8**:173.

31. Whitchurch CB, Tolker-Nielsen T, Ragas PC, Mattick JS: **Extracellular DNA required for bacterial biofilm formation**. *Science* 2002, **295**:1487.

32. Kadurugamuwa JL, Beveridge TJ: **Virulence factors are released from Pseudomonas aeruginosa in association with membrane vesicles during normal growth and exposure to gentamicin: a novel mechanism of enzyme secretion**. *J Bacteriol* 1995, **177**:3998–4008.

33. Muto Y, Goto S: **Transformation by extracellular DNA produced by Pseudomonas aeruginosa**. *Microbiol Immunol* 1986, **30**:621–628.

34. Zafra O, Lamprecht-Grandio M, de Figueras CG, Gonzalez-Pastor JE: **Extracellular DNA release by undomesticated Bacillus subtilis is regulated by early competence**. *PLoS One* 2012, **7**, e48716.

35. Lafer EM, Valle RP, Moller A, Nordheim A, Schur PH, Rich A, Stollar BD: **Z-DNA-specific antibodies in human systemic lupus erythematosus**. *J Clin Invest* 1983, **71**:314–321.

36. Sibley JT, Lee JS, Decoteau WE: **Left-handed "Z" DNA antibodies in rheumatoid arthritis and systemic lupus erythematosus**. *J Rheumatol* 1984, **11**:633–637.

37. Zhang T, Yin C, Boyd DF, Quarato G, Ingram JP, Shubina M, Ragan KB, Ishizuka T, Crawford JC, Tummers B, Rodriguez DA, Xue J, Peri S, Kaiser WJ, Lopez CB, Xu Y, Upton JW, Thomas PG, Green DR, Balachandran S: **Influenza virus Z-RNAs induce ZBP1-mediated necroptosis**. *Cell* 2020, **180**:1115–1129 e13.

38. Buzzo JR, Devaraj A, Gloag ES, Jurcisek JA, Robledo-Avila F, Kesler T, Wilbanks K, Mashburn-Warren L, Balu S, Wickham J, Novotny LA, Stoodley P, Bakaletz LO, Goodman SD: **Z-form extracellular DNA is a structural component of the bacterial biofilm matrix**. *Cell* 2021, **184**:5740–5758 e17.

39. Schwartz K, Ganesan M, Payne DE, Solomon MJ, Boles BR: **Extracellular DNA facilitates the formation of functional amyloids in Staphylococcus aureus biofilms**. *Mol Microbiol* 2016, **99**:123–134.

40. Tayeb-Fligelman E, Tabachnikov O, Moshe A, Goldshmidt-Tran O, Sawaya MR, Coquelle N, Colletier JP, Landau M: **The cytotoxic Staphylococcus aureus PSMalpha3 reveals a cross-alpha amyloid-like fibril**. *Science* 2017, **355**:831–833.

41. Domenech M, Garcia E, Prieto A, Moscoso M: **Insight into the composition of the intercellular matrix of Streptococcus pneumoniae biofilms**. *Environ Microbiol* 2013, **15**:502–516.

42. Hu W, Yang Z, Lux R, Zhao M, Wang J, He X, Shi W: **Direct visualization of the interaction between pilin and exopolysaccharides of Myxococcus xanthus with eGFP-fused PilA protein**. *FEMS Microbiol Lett* 2012, **326**:23–30.

43. Gustave JE, Jurcisek JA, McCoy KS, Goodman SD, Bakaletz LO: **Targeting bacterial integration host factor to disrupt biofilms associated with cystic fibrosis**. *J Cyst Fibros* 2013, **12**:384–389.

44. Novotny LA, Amer AO, Brockson ME, Goodman SD, Bakaletz LO: **Structural stability of Burkholderia cenocepacia biofilms is reliant on eDNA structure and presence of a bacterial nucleic acid binding protein**. *PLoS One* 2013, **8**, e67629.

45. Devaraj A, Justice SS, Bakaletz LO, Goodman SD: **DNABII proteins play a central role in UPEC biofilm structure**. *Mol Microbiol* 2015, **96**:1119–1135.

46. Devaraj A, Buzzo JR, Mashburn-Warren L, Gloag ES, Novotny LA, Stoodley P, Bakaletz LO, Goodman SD: **The extracellular DNA lattice of bacterial biofilms is structurally related to Holliday junction recombination intermediates**. *Proc Natl Acad Sci U S A* 2019, **116**:25068–25077.

47. Rieber M, Contreras CE, Rieber MS, Bianco NE: **DNA-protein complex and a large DNA in SLE cryoprecipitates**. *Clin Exp Immunol* 1986. Novel, **66**:61–67.

48. Pisetsky DS: **The central role of nucleic acids in the pathogenesis of systemic lupus erythematosus**. *F1000Res* 2019, **8**.

49. Biesecker SG, Nicastro LK, Wilson RP, Tukel C: **The functional amyloid curli protects Escherichia coli against complement-mediated bactericidal activity**. *Biomolecules* 2018, **8**.

50. Nicastro L, Tukel C: **Bacterial amyloids: the link between bacterial infections and autoimmunity**. *Trends Microbiol* 2019, **27**(11):954–963.

51. Rapsinski GJ, Wynosky-Dolfi MA, Oppong GO, Tursi SA, Wilson RP, Brodsky IE, Tukel C: **Toll-like receptor 2 and NLRP3 cooperate to recognize a functional bacterial amyloid, curli**. *Infect Immun* 2015, **83**:693–701.

52. Tukel C, Nishimori JH, Wilson RP, Winter MG, Keestra AM, van Putten JP, Baumler AJ: **Toll-like receptors 1 and 2 cooperatively mediate immune responses to curli, a common amyloid from enterobacterial biofilms**. *Cell Microbiol* 2010, **12**:1495–1505.

53. Tukel C, Raffatellu M, Humphries AD, Wilson RP, Andrews-Polymenis HL, Gull T, Figueiredo JF, Wong MH, Michelsen KS, Akcelik M, Adams LG, Baumler AJ: **CsgA is a pathogen-associated molecular pattern of Salmonella enterica serotype Typhimurium that is recognized by Toll-like receptor 2**. *Mol Microbiol* 2005, **58**:289–304.

54. De Luca F, Shoenfeld Y: **The microbiome in autoimmune diseases**. *Clin Exp Immunol* 2019, **195**:74–85.

55. Fine RL, Mubiru DL, Kriegel MA: **Friend or foe? Lactobacillus in the context of autoimmune disease**. *Adv Immunol* 2020, **146**:29–56.

56. Battaglia M, Garrett-Sinha LA: **Bacterial infections in lupus: roles in promoting immune activation and in pathogenesis of the disease**. *J Transl Autoimmun* 2021, **4**, 100078.

57. Torres-Ruiz J, Barrera-Vargas A, Ortiz-Hernandez R, Alcocer-Varela J, Ponce-de-Leon A, Gomez-Martin D: **Microbiological and immunological profile of patients with severe lupus flares related to bloodstream infections: a retrospective cohort study**. *Lupus* 2018, **27**:312–318.

58. Ceccarelli F, Perricone C, Olivieri G, Cipriano E, Spinelli FR, Valesini G, Conti F: **Staphylococcus aureus nasal carriage and autoimmune diseases: from pathogenic mechanisms to disease susceptibility and phenotype**. *Int J Mol Sci* 2019, **20**.

59. Chowdhary VR, Tilahun AY, Clark CR, Grande JP, Rajagopalan G: **Chronic exposure to staphylococcal**

superantigen elicits a systemic inflammatory disease mimicking lupus. *J Immunol* 2012, **189**:2054–2062.

60. Jung JY, Suh CH: **Infection in systemic lupus erythematosus, similarities, and differences with lupus flare**. *Korean J Intern Med* 2017, **32**:429–438.

61. Huang CF, Chen PL, Liu MF, Lee CC, Lee NY, Chang CM, Lee HC, Wu CJ, Ko WC: **Nontyphoidal Salmonella bacteremia in patients with connective tissue diseases**. *J Microbiol Immunol Infect* 2012, **45**:350–355.

62. Gerona JG, Navarra SV: **Salmonella infections in patients with systemic lupus erythematosus: a case series**. *Int J Rheum Dis* 2009, **12**:319–323.

63. Wu KC, Yao TC, Yeh KW, Huang JL: **Osteomyelitis in patients with systemic lupus erythematosus**. *J Rheumatol* 2004, **31**: 1340–1343.

64. Nockher WA, Wigand R, Schoeppe W, Scherberich JE: **Elevated levels of soluble CD14 in serum of patients with systemic lupus erythematosus**. *Clin Exp Immunol* 1994, **96**:15–19.

65. Mu Q, Zhang H, Luo XM: **SLE: another autoimmune disorder influenced by microbes and diet?** *Front Immunol* 2015, **6**:608.

66. Sirobhushanam S, Parsa N, Reed TJ, Berthier CC, Sarkar MK, Hile GA, Tsoi LC, Banfield J, Dobry C, Horswill AR, Gudjonsson JE, Kahlenberg JM: **Staphylococcus aureus colonization is increased on lupus skin lesions and is promoted by IFN-mediated barrier disruption**. *J Invest Dermatol* 2020, **140**:1066–10674 e4.

67. Chen X, Taylor DW, Fowler CC, Galan JE, Wang HW, Wolin SL: **An RNA degradation machine sculpted by Ro autoantigen and noncoding RNA**. *Cell* 2013, **153**:166–177.

68. Greiling TM, Dehner C, Chen X, Hughes K, Iniguez AJ, Boccitto M, Ruiz DZ, Renfroe SC, Vieira SM, Ruff WE, Sim S, Kriegel C, Glanternik J, Chen X, Girardi M, Degnan P, Costenbader KH, Goodman AL, Wolin SL, Kriegel MA: **Commensal orthologs of the human autoantigen Ro60 as triggers of autoimmunity in lupus**. *Sci Transl Med* 2018, **10**.

69. Hevia A, Milani C, Lopez P, Cuervo A, Arboleya S, Duranti S, Turroni F, Gonzalez S, Suarez A, Gueimonde M, Ventura M, Sanchez B, Margolles A: **Intestinal dysbiosis associated with systemic lupus erythematosus**. *mBio* 2014, **5**:e01548. 14.

70. * Azzouz D, Omarbekova A, Heguy A, Schwudke D, Gisch N, Rovin BH, Caricchio R, Buyon JP, Alekseyenko AV, Silverman GJ: **Lupus nephritis is linked to disease-activity associated expansions and immunity to a gut commensal**. *Ann Rheum Dis* 2019, **78**:947–956.

Patients with SLE had an overall 5-fold greater representation of *Ruminococcus gnavus (RG)* of the *Lachnospiraceae* family. Anti-RG antibodies correlated directly with SLEDAI score and antinative DNA levels, but inversely with C3 and C4. These antibodies were primarily against antigen(s) in an *RG* strain-restricted pool of cell wall lipoglycans.

71. * Bagavant H, Araszkiewicz AM, Ingram JK, Cizio K, Merrill JT, Arriens C, Guthridge JM, James JA, Deshmukh US: **Immune response to Enterococcus gallinarum in lupus patients is associated with a subset of lupus-associated autoantibodies**. *Front Immunol* 2021, **12**, 635072.

Anti-*E. gallinarum* IgG antibodies were measured in banked serum samples from SLE patients and healthy controls in the Oklahoma Cohort for Rheumatic Diseases. Higher anti-*E. gallinarum* titers were significantly associated with the presence of anti-dsDNA and anti-Sm autoantibodies.

72. Manfredo Vieira S, Hiltensperger M, Kumar V, Zegarra-Ruiz D, Dehner C, Khan N, Costa FRC, Tiniakou E, Greiling T, Ruff W, Barbieri A, Kriegel C, Mehta SS, Knight JR, Jain D, Goodman AL, Kriegel MA: **Translocation of a gut pathobiont drives autoimmunity in mice and humans**. *Science* 2018, **359**:1156–1161.

73. Pancewicz SA, Rutkowski R, Rutkowski K, Zajkowska JM, Kondrusik M: **[Immunopathology of Lyme arthritis]**. *Pol Merkur Lek* 2007, **23**:141–144.

74. Trollmo C, Meyer AL, Steere AC, Hafler DA, Huber BT: **Molecular mimicry in Lyme arthritis demonstrated at the single cell level: LFA-1 alpha L is a partial agonist for outer surface protein A-reactive T cells**. *J Immunol* 2001, **166**:5286–5291.

75. Elkington P, Tebruegge M, Mansour S: **Tuberculosis: an infection-initiated autoimmune disease?** *Trends Immunol* 2016, **37**:815–818.

76. Chodisetti SB, Rai PK, Gowthaman U, Pahari S, Agrewala JN: **Potential T cell epitopes of Mycobacterium tuberculosis that can instigate molecular mimicry against host: implications in autoimmune pathogenesis**. *BMC Immunol* 2012, **13**:13.

77. Maertzdorf J, Weiner 3rd J, Mollenkopf HJ, Network TB, Bauer T, Prasse A, Muller-Quernheim J, Kaufmann SH: **Common patterns and disease-related signatures in tuberculosis and sarcoidosis**. *Proc Natl Acad Sci U S A* 2012, **109**: 7853–7858.

78. Wu T, Tanguay RM: **Antibodies against heat shock proteins in environmental stresses and diseases: friend or foe?** *Cell Stress Chaperones* 2006, **11**:1–12.

79. Tishler M, Shoenfeld Y: **Anti-heat-shock protein antibodies in rheumatic and autoimmune diseases**. *Semin Arthritis Rheum* 1996, **26**:558–563.

80. Grando K, Nicastro LK, Tursi S, de Anda J, Lee EY, Wong GCL, Tukel C: **Phenol-soluble modulins from Staphylococcus aureus biofilms form complexes with DNA to drive autoimmunity**. *Front Cell Infect Microbiol* 2022, **12**:884065.

81. Zasloff M: **Antimicrobial peptides of multicellular organisms**. *Nature* 2002, **415**:389–395.

82. Lee EY, Zhang C, Di Domizio J, Jin F, Connell W, Hung M, Malkoff N, Veksler V, Gilliet M, Ren P, Wong GCL: **Helical antimicrobial peptides assemble into protofibril scaffolds that present ordered dsDNA to TLR9**. *Nat Commun* 2019, **10**: 1012.

83. Di Domizio J, Dorta-Estremera S, Gagea M, Ganguly D, Meller S, Li P, Zhao B, Tan FK, Bi L, Gilliet M, Cao W: **Nucleic acid-containing amyloid fibrils potently induce type I interferon and stimulate systemic autoimmunity**. *Proc Natl Acad Sci U S A* 2012, **109**:14550–14555.

84. Lee EY, Srinivasan Y, de Anda J, Nicastro LK, Tukel C, Wong GCL: **Functional reciprocity of amyloids and antimicrobial peptides: rethinking the role of supramolecular assembly in host defense, immune activation, and inflammation**. *Front Immunol* 2020, **11**:1629.

85. Peschel A, Otto M: **Phenol-soluble modulins and staphylococcal infection**. *Nat Rev Microbiol* 2013, **11**:667–673.

86. Schmidt NW, Jin F, Lande R, Curk T, Xian W, Lee C, Frasca L, Frenkel D, Dobnikar J, Gilliet M, Wong GC: **Liquid-crystalline ordering of antimicrobial peptide-DNA complexes controls TLR9 activation**. *Nat Mater* 2015, **14**:696–700.

87. Lee EY, Takahashi T, Curk T, Dobnikar J, Gallo RL, Wong GCL: **Crystallinity of double-stranded RNA-antimicrobial peptide complexes modulates toll-like receptor 3-mediated inflammation**. *ACS Nano* 2017, **11**:12145–12155.

88. * de Mello LR, Porosk L, Lourenco TC, Garcia BBM, Costa CAR, Han SW, de Souza JS, Langel U, da Silva ER: **Amyloid-like self-assembly of a hydrophobic cell-penetrating peptide and its use as a carrier for nucleic acids**. *ACS Appl Bio Mater* 2021, **4**: 6404–6416.

Hydrophobic cell penetrating peptide can self-assemble into amyloid-like fibers with β-sheet structure and chaperone dsDNA into the cell.

89. * Chen X, Yang X, de Anda J, Huang J, Li D, Xu H, Shields KS, Dzunkova M, Hansen J, Patel IJ, Yee EU, Golenbock DT, Grant MA, Wong GCL, Kelly CP: **Clostridioides difficile toxin A remodels membranes and mediates DNA entry into cells to activate toll-like receptor 9 signaling**. *Gastroenterology* 2020, **159**:2181–21892 e1.

Toxin A (TcdA) from *Clostridiodes difficile* can associate with dsDNA and induce cytokine production via toll-like receptor 9 (TLR9). Peptide fragments from this enterotoxin can form pro-inflammatory nanocrystalline structures with inter-dsDNA optimal for TLR9 activation.

90. Di Domizio J, Zhang R, Stagg LJ, Gagea M, Zhuo M, Ladbury JE, Cao W: **Binding with nucleic acids or glycosaminoglycans converts soluble protein oligomers to amyloid**. *J Biol Chem* 2012, **287**:736–747.