A wide range of molecules in plants and animals have the capacity to form net-like structures to trap pathogens. In concert with antimicrobial compounds, these aggregates can become efficient killing machines. In other instances, as with the intracellular septins, such structures may constrain pathogenic organisms and direct them to an autophagic tryst with deadly lysosomes. Most curiously, a meshwork of proteins in the mosquito gut appears to protect luminal bacteria from immune cells. Several recent studies delve into the intricacies of net formation and its role in microbial containment.

The remarkable powers of a certain fictional superhero derive from his ability to enmesh dangerous malfeasants in his synthetic webs. Unlike his arachnid counterparts, thankfully, he exhibits no culinary interests in the trapped victims. It is an effective foil, and one that diverse plants and animals have employed to ensnare harmful pathogens. A range of molecules have the ability to form such webs, and it is likely that many more will be found. By far, the best-studied are the extracellular traps (ETs) generated by dying polymorphonuclear neutrophils (NETs), eosinophils and mast cells. These cells of the granulocyte family contain specialized vesicles or “granules” with distinct toxic payloads. Neutrophils are well known for their phagocytic prowess, and the engulfed pathogens typically coalesce with toxic vesicles and meet a gruesome end. While phagocytosis occurs within minutes, NET formation takes between 2–4 h; it is not known if these are alternate fates, or if the same neutrophil can employ both strategies.

NETs are not random tangles, and their formation is not a happenstance event. They are comprised of ~17 nm-wide smooth filaments decorated with granular proteins to form ~50 nm-wide globular domains and, when fully hydrated, form cloud-like masses, emerging like large ghosts from dead neutrophils. Studded with antimicrobial compounds, they form a lethal and extended trap, an enormous jellyfish with extended arms.

Many signals, including pathogen-associated molecular patterns, can trigger NET formation. Neutrophils so triggered undergo NETosis, a unique type of cell death characterized by an initial dissolution of internal membranes. Nuclear and granular membranes disintegrate, the cells round up, the cell membrane breaks and the NETs—primarily a conglomerate of nuclear and granular components, but few cytoplasmic molecules—are released.

Some downstream events following receptor engagement include protein kinase C activation, signaling via the Raf-MEK-ERK and Rac2 pathways, the formation of reactive oxygen radicals by NADPH oxidase (phagocytic oxidase or PHOX) and migration of myeloperoxidase and the protease, neutrophil elastase, from granules. Statins and other inhibitors of the sterol synthetic pathway enhance NET formation and bacterial clearance, though they limit neutrophil phagocytosis and oxidative burst. Genetic defects in these pathways usually lead to impaired NET production and increased susceptibility to infections, although, since such mutations often have pleiotropic effects, it has been difficult to attribute susceptibility entirely to impaired NET formation.

Patients with chronic granulomatous disease (CGD), for instance, harbor...
mutations that affect NADPH oxidase function and have heightened susceptibility to infections since their neutrophils are defective for phagocytosis and NET formation. An indirect validation of the role of NETs in human infections came from a CGD patient with severe pulmonary aspergillosis. Reinstatement of NADPH oxidase activity via gene therapy restored NET formation and the consequent elimination of Aspergillus conidia and hyphae resulted in a rapid cure. Since fungal hyphae are too large to be phagocytosed, the study strongly implicates a role for NETs in fighting infections.

A completely different type of structure specialized in pathogen entrapment are the “nanonets” formed by the intestinal human α-defensin 6 (HD6).1 HD5 and HD6 are secreted by paneth cells, and some Crohn disease patients are deficient for these molecules. Despite its poor antimicrobial activity compared with HD5, HD6 inhibited invasion of S. typhimurium and Yersinia enterocolitica into cultured intestinal epithelial cells, and this property was dependent on a key histidine residue (H27, other human α-defensins have an amino acid residue at the corresponding position). Although human HD6-expressing transgenic mice challenged with Salmonella typhimurium and Yersinia enterocolitica had similar bacterial burdens in the gut lumen compared with infected wild-type animals, the bacteria were trapped in HD6 nanonets in the former. Correspondingly, the transgenic animals had lower levels of bacteria in Peyer’s patches and spleen. The authors of this study propose a model whereby HD6 binding to bacterial surface proteins, such as flagella, triggers the assembly of nanonets that contain the microbes. The nets formed by the host are not invariably harmful to resident microbes. Midgut epithelial cells of the Anopheles mosquito are separated from the blood meal and gut microflora by a mucin layer and a semipermeable peritrophic matrix made of chitin polymers.2 Two enzymes, immunomodulatory peroxidase (IMPer) and dual oxidase (duox), catalyze the formation of dityrosine linkages between matrix proteins to form a network that limits the reach of immune cells into the gut lumen and protects the microbiota. IMPer secretion is stimulated by a blood meal and is required for the survival of luminal bacteria. In insects depleted for IMPer via double-stranded RNA dependent silencing, luminal bacteria—as well as introduced Plasmodium berghei (tropical malarial parasite)—numbers were significantly reduced. In this case, the host appears to provide a “privileged site” to the resident luminal population. A recent report elegantly demonstrated the formation of an intracellular network of proteins that appear to play a role in autophagy. Interfered molecules of septin, a family of proteins involved in a range of cellular processes including cell division and cytoskeletal dynamics, were shown to trap intracytosolic Shigella flexneri in tight-fitting “cages.” The formation of the septin rings was dependent on actin polymerization, required myosin II activation and was interwoven with the process of autophagy. At any given time, 15–30% of the bacteria were contained in septin cages, thus restrained from cell-to-cell spread. In contrast, septin-free bac teria sprouted actin tails and zipped around unbound.

It is striking to note that many of the molecules now recognized for their ability to form nets are better known for their other functions in cells. It is conceivable that molecular tangles can wreak much havoc in living systems, but evolution has selected for several that appear to provide distinct advantages. So, even as cells tackled the incredible challenge of packaging nearly six feet of DNA within the confines of their nuclei, evolution has selected for the molecular version of a jack-in-the-box, popping out those parasitic cages to death. Disclosure of Potential Conflicts of Interest No potential conflicts of interest were disclosed.

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