Of Amoebae and Men: Extracellular DNA Traps as an Ancient Cell-Intrinsic Defense Mechanism

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Since the discovery of the formation of DNA-based extracellular traps (ETs) by neutrophils as an innate immune defense mechanism (1), hundreds of articles describe the involvement of ETs in physiological and pathological human and animal conditions [reviewed in Ref. (2), and the previous Frontiers Research Topic on NETosis: http://www.frontiersin.org/books/NETosis_At_the_Intersection_of_Cell_Biology_Microbiology_and_Immunology/195]. Interestingly, a few reports reveal that ETs can be formed by immune cells of more ancient organisms, as far back as the common ancestor of vertebrates and invertebrates (3). Recently, we reported that the Sentinel cells of the multicellular slug of the social amoeba Dictyostelium discoideum also produce ETs to trap and kill slug-invading bacteria [see Box 1; and Figure 1 Ref. (4)]. This is a strong evidence that DNA-based cell-intrinsic defense mechanisms emerged much earlier than thought, about 1.3 billion years ago. Amazingly, using extrusion of DNA as a weapon to capture and kill uningestable microbes has its rationale. During the emergence of multicellularity, a primitive innate immune system developed in the form of a dedicated set of specialized phagocytic cells. This professionalization of immunity allowed the evolution of sophisticated defense mechanisms including the sacrifice of a small set of cells by a mechanism related to NETosis. This altruistic behavior likely emerged in steps, starting from the release of “dispensable” mitochondrial DNA by D. discoideum Sentinel cells. Grounded in this realization, one can anticipate that in the near future, many more examples of the invention and fine-tuning of ETs by early metazoan ancestors will be identified. Consequently, it can be expected that this more complete picture of the evolution of ETs will impact our views of the involvement and pathologies linked to ETs in human and animals.

Keywords: amoebozoa, Dictyostelium, NOX, neutrophil extracellular traps, evolution, unicellular eukaryotes, multicellularity, innate immunity

During early evolution of multicellularity, when autonomous eukaryotic single-cell hosts were encountering prokaryotes, they either phagocytosed them as food or moved away to avoid being infected. However, when multicellular organisms evolved, they had to face more directly a serious problem, namely, infection of only parts or tissues of the organism. One solution is what happens in slugs of D. discoideum, in which invading bacteria are trapped by patrolling S cells that are subsequently shed behind during slug migration, keeping the multicellular structure free from infection (4, 8). The phagocytes in higher animals and men follow similar strategies to circumscribe the infection. For example, patrolling neutrophils catch the invaders and commit suicide, being finally
Box 1 | Dictyostelium discoideum as a unique model to study evolution of innate immunity

The social amoeba *Dictyostelium discoideum* belongs to the Amoebozoa, a sister group to the animals and fungi that branched after the divergence of plants (5, 6). The life cycle of *D. discoideum* comprises two major stages, a single-celled amoeboid stage and a “social” facultative multicellular stage. During the former, amoebae feed on bacteria and yeasts by phagocytosis, a biological process extremely well conserved in evolution and essentially shared between protozoan phagocytes and phagocytes of the animal innate immune system (7). These features make this genetically tractable organism a unique model to study the function of specific genes involved in the early evolution of innate immunity and the emergence of multicellularity.

**FIGURE 1 | Amoeba phagocytes and Sentinel cells capture and kill bacteria.** In the soil, solitary *D. discoideum* cells feed on bacteria, and starvation induces a developmental program, in which around 100,000 amoebae aggregate to form a migrating multicellular slug, followed by terminal differentiation and generation of fruiting bodies (28). During the migrating slug stage, only a few specialized cells, namely Sentinel (S) cells, keep their original phagocytic capacity and circulate through the slug to capture and kill invading microbes, functioning as a primitive innate immune system at the emergence of multicellular organism (8). In addition, the phagocytic S cells are constantly generated and sloughed off as the slug migrates. Our recent discovery showed that the S cells in the migrating slug of *D. discoideum* can produce extracellular DNA traps in a process that depends on production of reactive oxygen species (ROS) by NOX enzymes. Interestingly, S cells appear to mainly use their mitochondrial DNA to build up ETs, dissociating trap formation from immediate cells death by NETosis. Our study revealed that ET formation is a widespread DNA-based host defense strategy that may have been present in the ancestor of metazoa and amoebozoa.
### TABLE 1 | The co-emergence of NOX enzymes and multicellularity might also correlate with the origin of DNA-based defense strategies.

| UniProt Mnemonic | Classification | Species | Number of NOX homologs | Multicellularity |
|------------------|---------------|---------|------------------------|------------------|
| HUMAN            | Homo sapiens  | 7       | YES                    |
| MOUSE            | Mus musculus  | 6       | YES                    |
| CHICK            | Galus galus   | 6       | YES                    |
| ANOCA            | Anolis carolinensis | 5 | YES                |
| XENTR            | Xenopus tropicalis | 6 | YES                |
| TETNG            | Tetradon nigrovindis | 4 | YES                |
| ORYLA            | Oreiza latipes | 5       | YES                    |
| DANRE            | Danio rerio   | 5       | YES                    |
| BRAFL            | Branchiostoma floridæ | 6 | YES                |
| CIOM              | Ciona intestinalis | 6 | YES                |
| AEDAE            | Aedes aegypti | 2       | YES                    |
| ANOCA            | Anopheles gambiae | 2 | YES                |
| MMOCE            | Drosophila melanogaster | 1 | YES                |
| PEDDC            | Pediculus humanus subsp. corporis | 1 | YES                |
| DAPPY            | Daphnia pulex | 5       | YES                    |
| KOSC             | Ixodes scapularis | 1     | YES                    |
| CAEBR            | Caenorhabditis briggsae | 1 | YES                |
| CAEEL            | Caenorhabditis elegans | 2 | YES                |
| NEMVE            | Nematostella vectensis | 3     | YES                |
| MONBE            | Monosiga brevicollis | 1 | Transition          |
| NEUCR            | Neocarpa crassa | 2       | YES                    |
| PODAN            | Podospora anserina | 2 | YES                |
| ASPTN            | Aspergillus terreus | 2 | YES                |
| YEAST            | Saccharomyces cerevisiae | 0 | NO                   |
| SCHPO            | Schizosaccharomyces pombe | 0 | NO                   |
| DIOCI            | Dictyostelium discoideum | 3 | Transition          |
| POLPA            | Polysphondylymum pallidum | 2 | YES                |
| ENTHI            | Entamoeba histolytica | 0 | NO                   |
| ARATH            | Arabidopsis thallana | 10      | YES                   |
| PHYPA            | Physcomitrella patens | 4     | YES                    |
| CHLRE            | Chlamydomonas reinhardtii | 0 | NO                   |
| MOPS             | Micromonas pusilla | 0       | NO                    |
| OSTU             | Ostreococcus lucimarinus | 0 | NO                   |
| CYME             | Cyanidioschyzon merolae | 0     | NO                   |
| PHATC            | Phaeodactylus tricornutum | 0 | NO                   |
| PHIT              | Phytodilium intestans | 2     | YES                    |
| TETTH            | Tetrahymena thermophila | 0 | NO                   |
| LEIMA            | Leishmania major | 0       | NO                    |
| TRYCC            | Trypanosoma cruzi | 0       | NO                   |
| NAEGR            | Naegleria gruberi | 2       | NO                   |
| AMMYMU           | Amycolatopsis mediterranei | 0 | NO                   |
| ACTMD            | Actinosynnema mirum | 0       | NO                   |
| TRUURR           | Truepera radiovictrix | 0 | NO                   |
| THASP            | Thauera sp. | 0       | NO                    |
| ECOLI            | Escherichia coli | 0       | NO                    |
| VIBF1            | Vibrio Fischeri | 0       | NO                    |
| BACSU            | Bacillus subtilis | 0       | NO                    |

Representative organisms from both eukaryotes and prokaryotes [see Ref. (18) for detailed presentation] were collected and organized by major branches in taxonomy. The number of NOX enzymes in each organism is indicated and color coded. Unicellular and multicellular organisms are indicated by a “NO” and “YES,” respectively. Two organisms that are at the transition between the two life forms or have both life forms are indicated as “Transition.” One exception is Naegleria gruberi, a single-celled organism well known for its capacity to transition from an amoeboid to a flagellated form. It is a free-living organism, but closely related to pathogenic, parasitic species. Therefore, it is plausible that the NOX gene of Naegleria might have been acquired from its host via horizontal gene transfer or that it derives from an organism that was at the transition to multicellularity, but lost this characteristic of multicellular organisms as it specialized to its environment. The discovery of NOX-dependent ET generation in the multicellular form of the amoeba D. discoideum, an organism that is at the transition to multicellularity, combined to the recognition of the apparent co-emergence of multicellularity and NOX enzymes indicate that the origin of ET formation might be traced back to the emergence of multicellular organisms. It also suggests that variants and diverse evolutions of DNA-based defense strategies might be identified in other organisms with functional NOX enzymes, both in primitive metazoans and organisms close to the transition to multicellularity.
that express NOX homologs can generate ROS as signaling molecules to trigger ET formation for host defense. Interestingly, the evolutionary time of emergence of experimentally confirmed ET formation, NOX function, and multicellularity coincide well, possibly indicating that ROS-dependent DNA-based host defenses played a critical role in the early evolution of multicellular organisms guarded by an innate immune system.

In the near future, DNA-based host defense strategies will certainly be identified in a growing number of organisms. We propose that their study will reveal the fundamental significance in the relationship between host organisms and their coexisting commensals and pathogens and bring conceptual importance to the evolutionary time of emergence of experimentally confirmed ETs as ancient defense mechanisms that might have played a critical role in the evolution of multicellular organisms, and we need more systematic approaches and a broader perspective to recognize the importance of ETs in host–commensal and host–pathogen interactions. We expect that more related studies in the future will keep up the excitement in this field of research.

**AUTHOR CONTRIBUTIONS**

XZ and TS designed the experiments and interpreted the results. XZ performed the experiments and TS wrote the manuscript.

**FUNDING**

The work in the TS lab was supported by the European Cooperation in Science and Technology (COST) Action BM1203/EU-ROS, the Swiss SEFRI-COST No. C13.0137, and multiple grants from the Swiss National Science Foundation.

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