Research Paper E-ISSN NO : 2455-295X | VOLUME : 5 | ISSUE : 1 | JANUARY 2019

OBTAINING PERFORINS BY CHORDATES PROVIDES MORE EVIDENCE FOR THE PROTOZOAN ORIGIN OF LYMPHOCYTES

ALAN S COULSON M.D. [1] | CYNTHIA MCLEMORE M.D. [2] | ANGELA F COULSON. B.A. [3]

[1] 105 CROSSWINDS DRIVE, HAMLET, N.C. 28345.

[2] COMPANY HEALTH/MOORE CLINIC, 109 MCCOY DR., HOFFMAN, N.C. 28347.

[3] 105 CROSSWINDS DRIVE, HAMLET, N.C. 28345.

ABSTRACT

When the Chordate phylum first evolved the initial species did not possess the ability to make perforin. This meant the were easy prey for many parasites, as perforin is a critical defence protein.

Then about 500 to 600 million years ago some remarkable events occurred, simultaneously: lymphocytes made a sudden appearance, the Chordate phylum obtained perforin, and adaptive immunity in which the major histocompatibility complex plays a central role developed. In addition, complex signaling between immunological cells also appeared, and a T-cell receptor antigen recognition system was established.

No satisfactory explanation has ever been offered to explain the sudden appearance of a whole new class of circulating cells, the lymphocytes, and the associated immunological tools and weapons; and the method of acquiring perforin is usually dismissed as being by "a mechanism of horizontal gene transfer".

We suggest that all these events are related in that a new species was created when a substantial part of the genome of a protozoan parasite fused with an oocyte precursor in a Chordate host, bringing with it the ability to make perforin, and the ability to undertake complex intercellular signaling in one fell swoop. This event would also explain the continued confinement of Perforin 1 to the NK and CTL lymphocytes, and by virtue of their superior immune protection, would also contribute to an explanation of the subsequent dominance of Chordate fish clades in the Devonian period.

KEYWORDS: LYMPHOCYTES, PROTOZOA, EVOLUTION, PERFORINS.

SUBJECT:

This article discusses the significance of the Chordate phylum obtaining perforin, and proposes a new method of acquiring genomic change, by the fusion of the DNA from two different species resulting in the creation of a new species.

FIG 1 HUMAN PERFORIN TYPE 1

Human Perforin1is a water soluble 65 kD protein that can, when the circumstances demand, undergo a calcium-dependent aggregation. In the process of forming a circle of 20 monomers, the protein changes its shape dramatically resulting in a "cookie-cutter" formation that can penetrate the membrane of the victim cell in a matter of seconds, resulting in a fatal tubular wound to the cell.

INTRODUCTION:

In a previous article we pointed to the similarity of amoebapore and granulysin as supportive evidence for the protozoan origin of lymphocytes, (Coulson and Couson, 2016). We would now like to extend this line of thinking to a similar, albeit more complex, chemical defense system, the perforins.

This notion is in agreement with Dzik's general conclusion that many defence mechanisms believed to be to be specific to the immune systems of advanced metzoans have been inherited from unicellular eukaryotic ancestors, (Dzik, 2010). Among these he lists signaling which is based on the TIR domain, which is indispensable for protists, and mammalian lysins, which are related to similar factors secreted by amoebae.

Perforins are members of an ancient domain of M ACPF proteins, Membrane Attack Complex/Perforin, conserved in bacteria, fungi, mammals and plants. It was originally identified and as being common to five complement

components and perforin; hence its name. The MAC family proteins and perforin are known to respond to an infecting pathogen by forming membrane-attack complexes (MACs), which create transmembrane channels through the cell membrane of the target cell that cause the death of the invading micro organism.

The MACPF domain consists of a central kinked, four stranded antiparallel beta sheet surrounded by alpha helices and beta strands, forming two structural segments. Overall the MACPF domain has a thin L-shaped appearance.

The modus operandi of perforins is replete with complex nanotechnology, starting with perforin monomers binding to the target membrane via their C2 domains, then using a bridging action between adjacent N-terminal MACPF domains, they form a ring, rather like the well rehearsed choreography of ballet dances. At this point, the protein molecules undergo conformational changes: two clusters of alpha-helices within each MACPF domain, rearrange their amino acid sequences into anti-parallel beta strands, so that the hydrophobic portion of the molecule can puncture the membrane, creating a lethal pore. (Law, et al, 2010) rather like a molecular cookie cutter. This complex protein reconfiguration is best seen on a video posted by Lukoyanova et al (2015) on YouTube. In many ways it resembles a complex Arabesque ballet move.

The lethal pore may result in apoptosis of the attacked cell in a number of different ways, including the injection of granulysin into the pore.

At this point we should mention the possible confusion in terminology of the perforins: peforin 2 is widely distributed in the Animal Kingdom, except for the early chordates, whereas perforin 1 is confined to the CTL lymphocytes of vertebrates, notwithstanding a report that it is also present in brain tissue,(Gasque, et al). We adopted the suggestion of Podack and Munson (2016), and agree with them on this critical distinction between perforins 1 and 2. It would seem appropriate to encourage more work on perforin 2, in view of its evolutionary importance.

As mentioned above, the Chordate phylum initially did not possess any type of perforin, and so must have acquired the genetic information in the course of its evolution. Some authors have considered "lateral transfer" to have been the most likely mechanism. (D'Angelo et al, 2012)

Similarly the Urochordate, Ciona intestinalis, lacks the key genes for adaptive immunity, such as the MHC genes, T-cell receptor genes, and the crucial RAG (recombination activating gene), (Azumi et al, 2003).

PRESENCE OF PERFORIN IN PROTOZOAN PARASITES

Examples of Protozoan parasites that have the ability to make perforin or a perforin-like protein include: Plasmodium berghei (Deligianni et al, 2013), Plasmodium falciparum (Wirthet al, 2015), Toxoplasma gondii, (Roiko and Carruthers, 2013), and(Kafsack et al, 2009).

A family of five related genes in the genome of the rodent malaria parasite Plasmodium yoellii, encode secreted proteins all bearing a single MACPF - like domain. Each protein is highly conserved among Plasmodium species. Although not expressed in the blood stages, one of the genes was significantly expressed in P. yoelii sporozoites and in particular in the micronemes, the apparatus intimately involved in host-cell infection, indicating that MACPF - like proteins may play an important role in parasite interactions with the mosquito vector, (Kaiser et al, 2004) In addition, the identification of of genes encoding proteins with MACPF domains in the genome s of Eimeria and Toxoplasma indicate that this domain is not restricted to Plasmodium, but may be encoded by an ancient gene family of the exclusively parasitic phylum Apicocomplexa.

SUGGESTED ALTERNATE MECHANISM TO LATERAL TRANSFER, OF OBTAINING PERFORIN

Most authors have dismissed the way that perforin was obtained by Chordates as most likely by lateral transfer, but in the relay race of evolution this would be a huge baton of DNA to transfer. Flajnik (2014), in his review of of the evolution of the immune system suggests there were three lineages coupled with a "Big Bang". This would also involve the transfer of huge amounts of genetic information in an orderly fashion, so unlike usual course of events. For example the MHC sites appearing on every cell in the body, would have to coincide with appearance of lymphocytes to use them, otherwise, individually, they would have no survival value.

Another, and in our opinion more likely, consideration would be the fusion of the genetic information of a protozoan parasite with the precursor of a chordate oocyte, resulting essentially a new species which would have the parasites now as protective endosymbionts and would additionally be armed with the critical defensive protein perforin 2 in all its cells. Current thinking is that perforin 1 evolved from perforin 2 by a process of gene duplication of MPEG1, (D'Angelo et al, 2012) Ikegami and Kaneko (1990) emphasized the importance of considering genetic fusion in theoretical studies on evolution and introduced an algorithm to facilitate its use. Subsequently, Wostemeyer et al (2016) described such a fusion parasitism between Parasitella parastica and its host Absidia glauca, resulting in the production of hybrid cells.

In the case of the ascidian, Ciona intestinalis, primordial germ cells arise on the degenerated mass of the resorbed tadpole tail, and assemble to form a discrete gonad rudiment. Okada and Yamamoto (1999) described the sequence during the differentiation of the gonad rudiment into the testis and ovary. In a few days the primordial germ cells assembled to form a solid slender body together with flattened somatic cells, before it moved from the area of the esophagus. All it would take at this point, would be the infection of these cells by a protozoan parasite and a genetic fusion process to produce a new species of chordate with perforins and the fundamentals of a future immune system. Parthenogenesis, a phenomenon still seen

in modern day sharks (Chapman et al, 2007)), would ensure the genetic stability of the new species of chordates.

This would be a similar situation to the infection by the Microsporidian Octosporea effeminans and its sex determining influence in the amphipod Gammarus duebeni, (Bulnheim and Vavra (1968).

Or even more likely, the original protozoan parasite resembled the apicocomplexan parasites, which are known to extensively modify their host cells to ensure their own survival. Toxoplasma parasites are known to regulate host cell survival pathways and can even block MHC -dependent antigen presentation of parasite epitopes to avoid cell-mediated responses. Theileria parasites are acknowledged to be the masters of host cell modulation because their presence immortalises the infected cell, and that multiple pathways are activated to induce host cell transformation. (Luder et al, 2009).

In the case of infection with Theileria parva, association of the schizont with the host cell nuclear spindle ensures that daughter host cells remain infected during cytokinesis. Schizont DNA synthesis occurs as the host cell enters mitosis, with data indicating subversion of lymphocyte signal transduction pathways. (Bishop et al, 2004).

Additionally, Gomez et al, (2010) have nicely summarized the mechanisms of gene expression in protozoan parasites suggesting they contain many of the canonical machineries employed by higher eukaryotes for the control of gene expression at different levels. This is hardly surprising really when one takes into consideration the fact that protozoa have had a billion years of practice in the art of cell signaling before the first chordates were conceived.

CONCLUSION

Before the arrival of perforin in the chordates, they were small creatures like Lancelets and Acorn Worms; among other things, the acquistion of perforin proved to be a real game changer. As Canestro et al (2003) wrote somewhat dramatically: "Early Chordates were gentle, peaceful grazers of the photosynthetic prokaryotes; however their descendants include rapacious vertebrates, with cunning nervous systems that out-wit hapless victims..."

As mentioned in an earlier paper (Coulson, 2013), studies on cell fusion have shown that cells from different species can be melded such that the nucleus of the host cell resides in the cytoplasm of a cell from a different animal, the final product taking the form of a chimera, or a genetic consubstantiality.

The concept of genetic fusion seems to be gaining traction in other areas as well; Wostemeyer et al, (2016a) describing "fusion parasitism" albeit in fungi, as a system between sexuality and parasitism. Later they poise the all important question: "should fusion parasitism be seen as endocytobiosis?" Much of the ensuing discussion is similar to the ones we have been having, as is the discussion in another paper, "Understanding the establishment of endosymbionts in protozoa..." (Wostemeyer et al (2016b).

This new paradigm in evolutionary genetics might also explain the apparent saltatory advances in the evolution of some animals, where there would be inexplicable "missing links", if the process of evolution was confined to single point mutations.

The clinical significance of the protozoan prehistory of lymphocytes means that in many cases protozoan parasites, however, cannot be completely eliminated by the immune system, resulting in chronic infections. Guillermo et al (2009). In fact there are documented cases where lymphocytes actually assist in the invasion of the host victim, notably Human African Trypanosomiasis (Coulson and McLemore 2017)

Since the human genome contains viral and lots of other pieces of seemingly useless intron causing material, (Crisp et al, 2015) it is not impossible that what was originally protozoan DNA is also incorporated in there.

REFERENCES

1. Azumi, K., De Santis, R., De Tomaso, A., Rigoutsos, I., Yoshizaki, F., et al. (2003). Genomic analysis of immunity in a Urochordate and the emergence of the vertebrate immune system: "wating for Godot", Immunogenetics, 55, p. 570 – 581.

2. Bishop, R., Musoke,A., Morzaria, M., et al . (2004). Theileria: intracellular protozoan parasites of wild and domestic ruminants transmitted by ixodid ticks. Parasitology 129 Suppl: S271 – 83.

3. Bulenheim, H. -P., and Vavra, J. (1968). Infection by the Microsporidian Octosporea effeminans Sp.N., and its sex determining influence in the Amphipod Gammarus duebeni. J of Parasitology, 54, p. 241 - 248

4. Canestro, C.,Bassham, S., and Postlethwait, J.H. (2003). Seeing chordate evolution through the Cianogenome sequence. Genome Biology, 4 p. 208. doi.org/10.1186/gb-203-4-3-208 accessed 21Dec 2018

5. Chapman, D.D., Shivji, M.S., Louids, E., et al (2007) Virgin birth in a hammerhead shark. Biol. Lett., 3, p.425 – 427.

6. Coulson, A. S., (2013). Evolution Concept: Possible Protozoan Ancestry For Lymphocytes. The Evolver, Newsletter of the Evolution SIG of British Mensa, 43, p. 11 – 18.

7. Coulson, A.S., and Coulson, A.F. (2016). Evolution of Lymphocytes from Protozoa: the Hypothesis is Supported by the similarity of Amoebapore and Granulysin. International Educational Scientific Research Journal, 2, p 15 – 16.

8. Coulson, A. S., and McLemore, C., W. (2017). Human African Trypanosomiasis: Possible Treatment

with Anti-lymphocyte Serum. International Educational Scientific Research Journal, 3, p 40 – 43.

9. Crisp, A., Boschetti, C., Perry, M., Tunnacliffe, A. and Micklem, G. (2015). Expression of multiple horizontally acquired genes is a hallmark of both vertebrate and invertebrate genomes. Genome Biology,16, p 50 - 63.

10. D'Angelo, M. E., Dunstone, M. A., Whisstock, J.C. et al (2012). Perforin evolved from a gene duplication of MPEG1, followed by a complex pattern of gene gain and loss within Euteleostomi. BMC Evolution Biol 12, p 59 doi.org/10.1186/1471-2148-12-59 accessed 21 Dec 2018.

11. Deligianni, E., Morgan, R.N., Bertucci, L., et al, (2013), A perforin-like protein mediates disruption of the erythrocyte membrane during egress of Plasmodium berghei male gametocytes. Cell Microbiol, 15 p 1438 – 55.

12. Dzik, J. M., (2010). The ancestry and cumulative evolution of immune reactions. Acta Biochimika Polonica, 57, p. 443-466

13. Flajnik, M. F., (2014). Re-evaluation of the Immunological Big Bang. Current Biol 24, doi.org/10.1016/j.cub.2014.09.070. Accessed 21 Dec 2018.

14. Gasque, P. Jones, J. Singhrao S.K., and Morgan, B. (1998) Identification of an Astrocyte Cell Population from Human Brain that Expresses Perforin, a Cytotoxic Protein Implicated in Immune Defense. J Exp Med., 187, p 451 – 460.

15. Gomez, C., Ramirez, M., E., Calixto-Galvez M., et al (2010). Regulation of Gene Expression in Protozoan Parasites. J of Biomed and Biotechnology,2010 (1110 – 7243):726045. DOI: 10.1155/2010/726045. accessed 11 Jan 2019.

16. Guillemo, L.,C., Pereira, W.F., De Meis J.,et al (2009). Targeting caspases in intracellular protozoan infections. Immunopharmacology and Immunotoxicology, 31 p 159 – 173.

17. Ikegami, T., and Kaneko, K. (1990). Genetic Fusion. Physical Review, 65, p 3352 – 3355.

18. Kafsack, B.,F., C., Pena J.D.O., Coppens, I., et al, (2009). Rapid Membrane Disruption by a Perforin-Like Protein Facilitates Parasite Exit from Host Cells. Science 323, p 530-533.

19. Kaiser, K., Camargo, N., Coppens, I., et al (2004). A member of a conserved Plasmodium protein family with membrane-attack complex/perforin (MACPF)-like domains localizes to the micronemes of sporozoites. Molecular & Biochem Pathology 133, p15 – 26.

20. Law, R. H. P., Lukoyanova, N., Vosoboinik, I., Carodoc-Davies, T.T. et al. (2010). The structural basis for membrane and pore formation by lymphocyte perforin. Nature, 468, p. 447 – 451.

21. Luder, C, J. K., Stanway R. R., Chaussepied M., et al (2009). Intracellular Survival of Apicocomplexan parasite and host cell modification. Int J for Parasitology. 39, p163 – 173.

22. Lukoyanova, N., Kondos, S. C., Farabella, I., Law, R.H.P., Reboul, C. F., Caracadoc-Davies, T. T., et al (2015). Conformational Changes during Pore Formation by the Perforin-Related Protein Pleurotolysin. PloS Biology http://dx.doi.org/10.1371/journal.pbi. http://www.youtube.com/watch? v=jaBoJWKXUs accessed on Jan 7, 2019.

23. McCormack, R., and Podack, E.R. (2015). Perforin-2/Mpeg and other pore-forming proteins throughout evolution. J Leukoc Biol, 98, p761-768.

24. Okada T. and Yamamoto, M. (1999). Differentiation of the gonad rudiment into ovary and testis in the solitary ascidian, Ciona intestinalis. Develop Growth Differ. 41 p 759 – 768.

25. Podack, E.R., and Munson, G. P. (2016). Killing of Microbes and Cancer by Immune System with Three Mammalian Pore-Forming Killer Proteins, Front. Immunol. https://doi.org/10.3389/fimmun.2016.00464. accessed on Nov27 2018.

26. Roiko, M. S., and Carruthers, V. B, (2013, Functional dissection of Toxoplasma gondii perforin-like protein 1 reveals a dual domain mode of membrane binding for cytolysis and parasite egress. J Biol Chem, 288 p.12 – 25.

27. Wirth, C.C,, Bennink, S., Scheuermayer, M., et al, (2015) Perforin-like protein PPLP4 is crucial for mosquito midgut infection by Plasmodium falciparum. Mol Biochem Parasitol 201, p 90 – 99.

28. Wostemeyer, J., Ellenberger,S., Schulz, E. et al (2016) Fusion parasitism between Parasitella parasitica and its host Absidia glauca: A system between sexuality and parasitism. Endocytobiosis and Cell Research, 27, p 24 – 32.

29. Wostemeyer, J., Siegmund, L., and Ellenberger, S. (2016) Understanding the establishment of endosymbionts in protozoa: Development of a laboratory model for Tetrahymena pyriiformis Endosymbiotic association in nature. Endocytobiosis and Cell Research, 27 p 11 – 15.