PHYTOHAEMAGGLUTININ RESPONSIVENESS BY SOME PROTOZOA PROVIDES FURTHER SUPPORT FOR THE HYPOTHESIS THAT LYMPHOCYTES EVOLVED FROM PROTOZOA

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ABSTRACT

Phytohaemagglutinin (PHA) is a lectin found in plants, especially certain legumes. It consists of two closely related proteins: PHA-E which agglutinates erythrocytes, and PHA-L which agglutinates white blood cells and also causes them to undergo blast cell transformation and subsequent mitosis. The way PHA causes these effects on T cells is still poorly defined, and it has no known evolutionary survival value. The 36 phyla that currently make up the Animal Kingdom were reviewed using the technique of Azzolina et al (1985); and only 4 other phyla were found to contain organisms or cells that responded to PHA: Protozoa, Annelida, Platyhelminths, and sponges. In the phylum Protozoa, a strong mitogenic action by PHA on free living soil amoebae has been described, although whether this phenomenon was the same as that occurring in lymphocytes still "remains to be clarified". In Bursaria truncatella the mean generation time was shortened, and in Paramecium aurelia a growth stimulus occurred in the G1 period. In the phylum Platyhelmithes, Dugesia dorotocephala was found to regenerate more rapidly in the presence of PHA. In the Phylum Annelida, PHA caused the transformation of some coelomocytes of the Lubricus terrestris. Protozoa evolved some 1.5 billion years ago, and Tunicates about 550 million years ago, while the earliest Phaseolus appeared much later, about 80 million years ago. Hence while the PHA effect of the bean proteins probably had no effect on the survival of the two animal phyla, it is possible that PHA and other lectins exerted a protective effect on the beans; as there is a distinct paucity of Protozoan parasites infecting plants (Peumans and Van Damme, 1995).

Another conclusion that might tentatively be drawn from the observation of the similarity of this singular PHA responsiveness, is that there is an evolutionary connection between the lymphocytes of the Chordates and some members of the Protozoan phylum.

KEYWORDS: Phytohaemagglutinin, Protozoa, Lymphocytes, Evolution.

FIGURE 1.

CAPTION FOR FIGURE 1: Crystal structure of PHA L;

PHA L is a tetramer of 4 L-type subunits held together by noncovalent forces. It is a selectin, a member of one of the important groups of lectins; a central channel is demonstrated. Mol wt 115,000 Da. Rectangular shape: dimensions 77 x 43 A

INTRODUCTION:

The structure of PHA-L is shown in FIGURE 1. (FIG 1 NEAR HERE) It is a lectin found in high concentrations in the red kidney bean Phaseolus vulgaris. Lectins are inceasingly being recognized as important large molecules that bind to a soluble carbohydrate or to a carbohydrate moiety, that is part of a glycoprotein or glycolipid, (Sharon and Lis, 1994). They typically agglutinate certain animal cells and/or precipitate glycoconjugates, (Movafagh et al, 2013).

PHA is an N-acetylgalactosamine/galactose sugar- specific lectin, with a wide variety of biological activities. In this article we would like to focus on its mitogenic properties. Current dogma is that the engagement of enough signaling components on the cell surface leads to a critical clustering that, in turn, initiates the calcium-dependent pathway to nuclear activation, (SharonandLis, 2004, and Lindahl-Kiessling,1972).

Previously it was thought that distortion of the receptor site provided the critical signal, (Coulson, 1975 and Hivroz and Saitakis, 2016). Nevertheless whichever method of activation proves to be the correct one, it is noteworthy that of all the cells in the vertebrate body PHA has this effect only on the lymphocyte population. As early as 1968, Caso (1968) showed that PHA had an inhibitory action on epithelioid cell lines and on fibroblasts in vitro. Sell and da Costa (2003) reported that PHA significantly reduced fibroblast proliferation, and at higher concentrations caused cell death.

PHA AND PROTOZOA

Zech (1966) showed that PHA shortened the generation time of Bursaria truncatella from 24 – 28 hours to about 20 hours. In the case of Stentor coeruleus, PHA initiated gtowth and division; and similarly Kovachev (1983) documented that PHA reduced the generation time of Paramecium caudatum. An optimal cconcentration of 50 micrograms/ml was noted.

According to Kovachev other authors had reported similar findings with other prtotozoa.

Csaba et al, (1983,) described the mitogenic effect of PHA on Tetahymena; and Agrell (1966) similarly described the mitogenic effect of phosphate and phytohaemagglutnin on free – living amoebae.

Dwyer (1974), descrbed a PHA binding site on Leishmania donovani; and Bose et al (1989), using fluorescein-conjugated preparations, demonstrated that two lectins, Concanavalin A and Phytohaemagglutinin P, could bind the free-living amoebae Acanthamoeba astronyxis, A.castellanii and Naegleria fowleri.

The major point of interest in most of these cases is that agglutination of the Protozoa was not described, and it did not seem to be a necessary preliminary before cell division. So one is left with the impression that the binding of PHA to the cell membrane is just a fluke of nature, one that occurs only twice more in the Animal Kingdom, in Chordate lymphocytes and in Annelida..

Despite the fact that PHA responsiveness has no known survival value in protozoa, it does illustrate the point that complex signal transduction occurs in protozoa in order to modulate fundamental cellular activities. In fact evidence presented by Renaud et al (2004) suggests that such transduction events are similar to those present in higher eukaryotes, and may simply have been conserved from their ancient origins. New and Wong, (1998), reached a similar conclusion, as did Eichinger et al, (2002).

PHA AND SPONGES

Richelle-Maurer et al, (2003), found that PHA promoted aggregation and the reorganization of cell suspensions derived from the marine sponge Xestspongia muta.

PHA AND COELOMOCYTES OF LUMBRICUS TERRESTIS

Toupin et al (1977) reported that some of the coelomocytes of Lumbricus terrestris transformed when exposed to PHA. They concluded that these results reinforced the existence in earthworms of a T-cell like function and perhaps receptors similar to those observed in vertebrates.

PHA AND PLANARIANS

It was reported by Ruiz et al, that PHA treament significantly reduced the regeneration time of the blastema tissue of the Planarian Dugesia dorotocephala.

PHA AND CHORDATE LYMPHOCYTES

There is a voluminous literature on the in vitro mitogenic effect of PHA on Chordate lymphocytes, starting with the Tunicates, (Raftos et al, 1991).

According to the fluid mosaic membrane model of Singer and Nicolson, (Nicolson, 1974), the lymphocyte cell membrane consists of proteins and glycoproteins floating in a lipid bilayer; and the most recent theory as to the way

PHA exerts its effect is by causing a clustering of specific receptors, including CD2 T cell receptors. After they become cross-linked, they trigger calcium-dependent pathways leading to nuclear activation, (Movafagh, 2011). Singh and Walia (2012) have developed a detailed diagram of the proposed mechanism of mitogenic stimulation, partly based on the work of Bertozzi and Kiessling, (2001).

Since this phenomenon has no known survival value it should also considered to be a fluke of nature. In fact under normal circumstances, in humans lectins have been reported to cause damage, including mass food poisoning, (Kumar et al, 2012).

DISCUSSION AND CONCLUSION

It seems to be a remarkable coincidence that responsiveness to PHA, an artificial in vitro, laboratory phenomenon, is found to exist in certain Protozoa and in Chordate T cells. It has no known evolutionary value to either of these cells, and additionally, the biological role of these lectins in natural circumstances 'is not clear', (Csaba, 2016).

The protective carbohydrate sheath, glycoconjugates, enclosing free-living Protozoa may be too thick in places to permit PHA to approach the receptors as easily as it does in lymphocytes, which reside in the protection of the blood stream. This may explain higher responsive rate in lymphocytes to PHA compared to amoeba for example.

The acquisition of PHA responsivenss could have been in 3 different ways; lateral transfer, or parallel evolution, or it could have been incidentally transferred when the original protozoan parasite became an endosymbiont. We favour the latter explanation as the possession of PHA responsiveness bestows no known survival advantage.

After many years of research the precise way PHA produces its effect remains unknown. When the exact nature of the PHA binding to the cell membrane, and the subsequent pathway to nuclear activation, are elucidated, we suspect there will be close similarities found in Protozoa and T cells. Until that time, we suggest that continued PHA responsiveness, as it was in the beginning, be considered as supportive evidence that protozoa evolved into valuable lymphocyte endosymbionts, even as we concede that this beggars the bigger question, viz,'why do both these cell types, chordate lymphocytes and protozoa continue to respond to PHA ?'

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