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Review

Immunomodulation by phosphocholine—Biosynthesis, structures and immunological implications of parasitic PC-epitopes

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ABSTRACT

Phosphocholine (PC) as a small haptenic molecule present on antigens of parasites can provoke various effects on immune cells leading to immunomodulation of the host's immune system. This immunomodulation not only allows long-term persistence but also prevents severe pathology due to down-regulation of cellular immune responses. Additionally, PC plays an important role for development and fertility of the parasites. To fully understand the mechanisms of immunomodulation the detailed knowledge of the biosynthesis of the PC-epitopes, their molecular structure and biological function has to be elucidated. The implication of parasite-specific transferases in the biosynthesis of the PC-epitopes and the sensitivity of parasites towards disruption of the choline metabolism offers new perspectives for the development of anti-parasitic drugs and therapies. Furthermore, the immunomodulation provoked by PC-epitopes preventing inflammatory reactions may be useful in the treatment of inflammatory diseases. This review summarizes the current knowledge on the biosynthesis of PC-epitopes, their structures and immunological implications.

1. Introduction

During evolution parasites have developed various mechanisms for the successful invasion and persistence in their hosts: camouflage by acquisition of host derived molecules, molecular mimicry by synthesizing structures related or identical to host biomolecules or immunomodulation by active interference with the hosts' immune reactions. One of the most sophisticated mechanisms of immunomodulation is the use of phosphocholine (PC). PC, a small haptenic molecule, is found in a wide variety of prokaryotic organisms, i.e. bacteria, and in eukaryotic parasites. Amongst multicellular parasites this small haptenic molecule is predominantly found not only in nematodes but also in protozoa. Nematode and protozoan infections are the most common cause of diseases in humans worldwide. It is estimated that on third of the world's population is infected with nematodes whereas the intraerythrocytic protozoan parasite *Plasmodium falciparum* is responsible for more than 500 million clinical cases of tropical malaria annually and up to 3 millions of infected people die (Golenser et al., 2006; Nacher et al., 2000). By immunomodulation nematode infections cause a chronic, long-lasting disease with adult worms able to survive within the hosts for up to 10 years (Houston and Harnett, 2004). Nematode infections are characterized by low mortality but high morbidity with a significant proportion of sufferers exhibiting severe health problems (e.g., *Brugia malayi*—severe skin lesions, *Wuchereria bancrofti*—elephantiasis and *Onchocerca volvulus*—eye damage and blindness) (Subramanian et al., 2004). Studies revealed that helminth infections increase susceptibility, but reduce the risk to develop reduce cerebral malaria (Nacher, 2001; Spiegel et al., 2003; Yoshida et al., 2000), probably due to immunomodulation. Furthermore, there is evidence that helminth infections (e.g. *Schistosoma mansoni*, *Trichinella spiralis*) can delay or prevent the onset of a wide range of autoimmune disorders (diabetes, asthma, allergic encephalomyelitis or rheumatoid arthritis) (Dunne and Cooke, 2005; Harnett and Harnett, 2006a; Maizels, 2005).

To fully understand the mechanisms of immunomodulation by PC the detailed knowledge of the biosynthesis of the PC-epitopes, their molecular structure and biological function has to be elucidated.

In multicellular parasites proteins and glycolipids have been found to be decorated with PC (Cipollo et al., 2004; Friedl et al., 2000; Grabitzki et al., 2008; Lochnit et al., 2000). However, despite intensive research in the past years, neither the donor for PC nor the respective PC-transferase has been identified so far for any parasite or free-living model system. Structural analyses of glycoconjugates derived from nematodes revealed the presence of nematode-specific glycosphingolipids of the arthro-series, carrying, in part, PC-substituents. PC-modified glycosphingolipids can be regarded as a phylogenetic marker for nematodes (Friedl et al.,

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2003; Lochnit et al., 1998). However, this modification seems to have a dual role for nematodes as it has been found to play an important role also in development and reproduction of nematodes as shown for the free-living model system *Caenorhabditis elegans*. RNAi experiments targeting the glycosphingolipid-biosynthesis and choline-metabolism resulted to a dramatic reduction of the offspring production. Furthermore, inhibitor studies with chemical inhibitors sustained the importance of PC-modification for nematode development (Houston et al., 2007; Lochnit et al., 2005). More recently, the first PC-substituted proteins from *P. falciparum* have been identified (Grabitzki et al., unpublished results).

PC-bearing components have been found to interfere with key proliferative signalling pathways in B- and T-cells, development of dendritic cells and macrophages and mast cell degranulation (Goodridge et al., 2001; Harnett and Harnett, 2001; Melendez et al., 2007; Whelan et al., 2000). These effects contribute to the observed low antibody and cytokine levels and impairment of lymphocyte proliferation (Couper et al., 2005; Dell et al., 1999; Goodridge et al., 2003; Harnett and Harnett, 2001; Harnett et al., 2004; Marshall et al., 2005). However, the anti-inflammatory potential of PC-antigens may be of clinical relevance for new concepts in the treatment of allergies and autoimmune diseases (Harnett et al., 2008).

This review summarizes the current knowledge on the biosynthesis, the structure and the immunological functions of PC-antigens with a focus on nematodes and protozoa.

2. Choline metabolism and biosynthesis of PC-epitopes

Extensive investigations in the past years have revealed four biosynthetic pathways (see Fig. 1) leading to the synthesis of choline metabolites in free-living and parasitic nematodes and in *P. falciparum*: the Kennedy-pathway with the conversion of choline to phosphocholine by choline kinases, the activation of phosphocholine to CDP-choline by CTP:phosphocholine cytidylyltransferases and the subsequent formation of phosphatidylcholine by diacylglycerol:cholinephosphotranferases; the Bremer–Greenberg-pathway comprising the stepwise methylation of phosphatidylethanolamine generated by decarboxylation of phosphatidylserine to phosphatidylcholine by phosphatidylethanolamine *N*-methyltransferases; the phosphoethanolamine-methyltransferase-pathway with the stepwise methylation of phosphoethanolamine to phosphocholine by phosphoethanolamine *N*-methyltransferases; and finally, the serine-decarboxylation-phosphoethanolamine-methylation-pathway starting from serine-decarboxylation with subsequent phosphorylation of ethanolamine.

Genome and experimental data suggest that all four pathways are present in *C. elegans*. Interestingly, for many enzymes involved in choline metabolism genome annotations showed the presence of multiple homologue enzymes in this model organism. This may reflect the importance of the choline metabolism for this nematode.

The stepwise methylation of phosphoethanolamine to phosphocholine in *C. elegans* is catalyzed by two *N*-methyltransferases. Phosphoethanolamine *N*-methyltransferase PMT-1 is responsible for the transfer of the first methyl-group from *S*-adenosyl methionine to phosphoethanolamine (Brendza et al., 2007), whereas PMT-2 catalyzes the subsequent transfer of the two other methyl-groups (Palavalli et al., 2006). RNAi experiments in *C. elegans* targeting PMT-1 and PMT-2 resulted in P0 sterility and larval arrest or lethality. Additionally, the existence of the Kennedy- and the Bremer–Greenberg-pathways has been demonstrated (Lochnit and Geyer, 2003). RNAi experiments targeting the diacylglycerol:choline phosphotransferase (CPT, F22E10.5) in *C. elegans* significantly reduced the PC-substitution of ES-62 as demonstrated by Western-blotting with the PC-specific antibody TEPC-15 (Houston et al., 2007) indicating an important role of the Kennedy-pathway for PC-modifications. In a large RNAi study targeting enzymes of the Kennedy- and Bremer–Greenberg-pathways significant reductions in fertility have been observed (Lochnit et al., 2005). These effects were even more dramatic when double-RNAi experiments targeting homologous enzymes were performed (see Fig. 2). This is a further indication for the importance of the choline metabolism for these organisms. Having multiple homologous enzymes of important pathways in the genome seems to be advantageous for evolutionary success.

P. falciparum uses the serine-decarboxylation-phosphoethanolamine-methylation-pathway (Pessi et al., 2005; Pessi et al., 2004) as well as the Kennedy-pathway (Choubey et al., 2006). A vital role for the choline kinase in trapping the essential nutrient choline in *P. falciparum* is discussed (Choubey et al., 2006).

The attachment of PC to glycosphingolipids and (glycol)proteins is still not fully elucidated. There is still a debate about the nature of the PC-donor(s) and the enzymes involved. So far, the PC-transferase is not identified and attempts to purify the enzymatic activity have failed.

The attachment of PC to proteins has been intensively studied for the excretory–secretory product ES-62 of *A. viteae*. PC addition was found to be an early event during intracellular processing. Cultivation of the parasite in the presence of tunicamycin, an inhibitor of *N*-glycosylation, resulted in partially blocked secretion, and inhibited incorporation of radiolabelled glucosamine and choline (Houston and Harnett, 1996). Further studies on the site and mechanism of PC-attachment revealed inhibitory effects of brefeldin A, an inhibitor of protein trafficking from the endoplasmic reticulum (ER) to the Golgi, 1-deoxynojirimycin, an inhibitor of α-glucosidase I activity in the ER and 1-deoxymannojirimycin, an inhibitor of α-mannosidase I in the cis-Golgi (Houston et al., 1997; Houston and Harnett, 1999a). Swainsonine, an inhibitor of α-mannosidase II in the medial Golgi had no effect. These results suggested that PC-attachment is a post-ER event, dependent on the generation of an acceptor structure $Man_5GlcNAc_3$ or $Man_3GlcNAc_3$ in the medial Golgi.

A phosphocholinetransferase activity, however, has been demonstrated *in vitro* for *A. viteae* and *C. elegans* microsomal preparations by formation of radiolabelled phosphatidylcholine, when [^{14}C]CDP-choline was used as donor (Houston and Harnett, 1999b; Lochnit and Geyer, 2003).

The first *in vivo* investigations on the biosynthesis of *A. viteae* ES-62 focusing on the biochemical pathway of choline synthesis failed to give any evidence for the necessity of CDP-choline, since CDP-choline added to the medium resulted in the labelling of phosphatidylcholine and sphingomyelin, but not of ES-62 (Houston and Harnett, 1999b). One explanation for this finding may be the inability of CDP-choline to reach the lumen of the Golgi where the transfer to *N*-acetylglucosamine (GlcNAc) residues is postulated to occur, whereas phosphatidylcholine biosynthesis takes place on the accessible, cytosolic side of the ER. Hexadecylphosphorylcholine, an inhibitor of CDP-choline synthesis, only showed some reduction in PC addition to ES-62. Blocking of choline kinase activity by cultivation of *A. viteae* in the presence of hemicholinium-3, however, resulted in the complete absence of PC from ES-62, as shown by autoradiography and Western-blotting. Although hemicholinium-3 is also known to block the uptake of choline into cells by competitive inhibition of the high affinity choline transporter, the authors provided data suggesting the dependence on choline kinase activity for the transfer of PC to ES-62-bound carbohydrates (Houston and Harnett, 1999b).

The first experimental indication for phosphatidylcholine as the donor of the PC-residues on *N*-glycans was demonstrated by Cipollo et al. (2004) using *C. elegans* microsomes. They used endo H-released oligosaccharides from ovalbumin as acceptor structures