Natural and Synthetic Glycan Arrays for Probing Interactions of the Innate and Adaptive Immune System with Zwitterionic Oligosaccharides

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MARIE CURIE ACTIONS

GlycoPar
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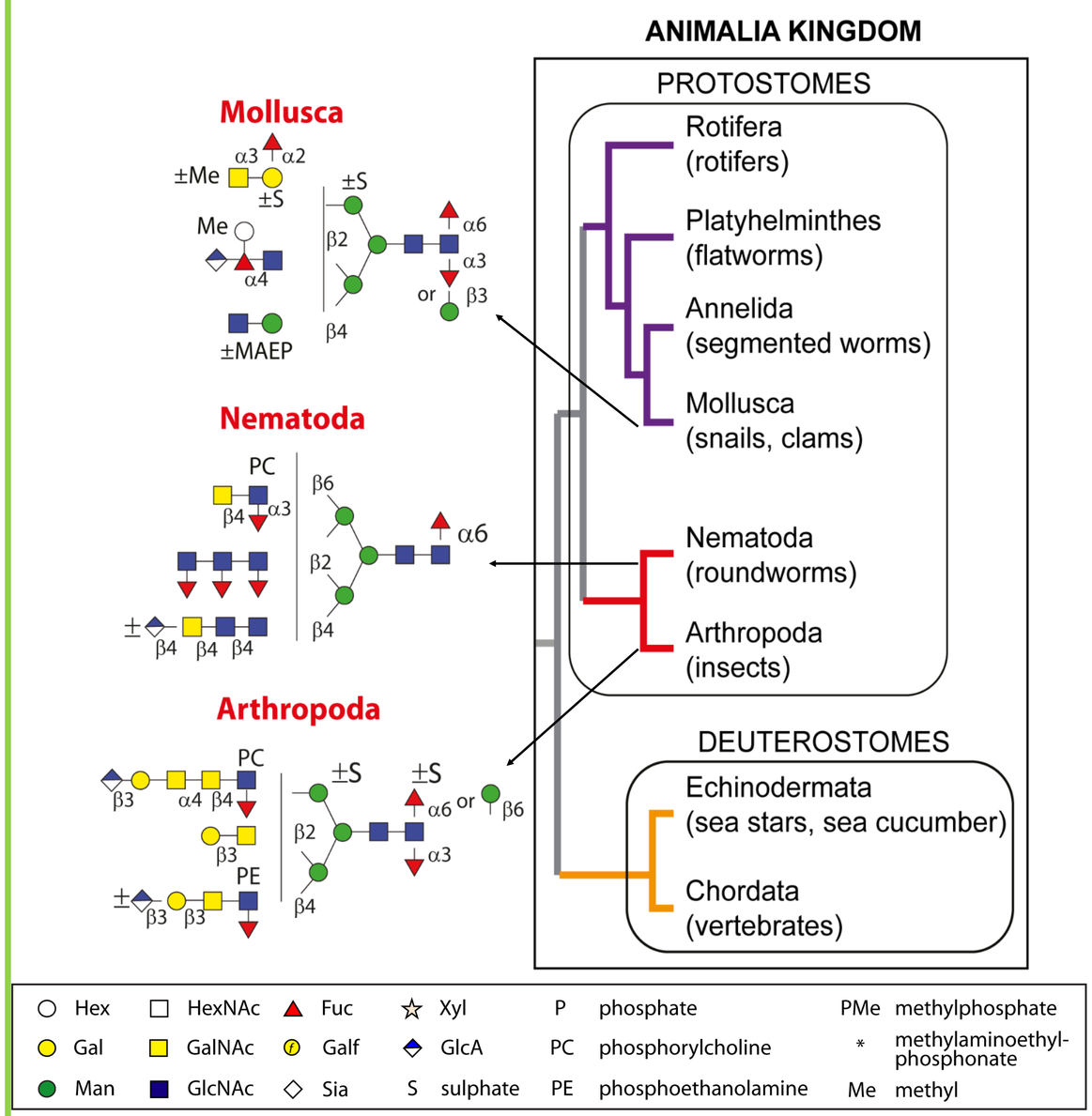
Glycans cover the surfaces of all cells and most secreted proteins from eukaryotes are also glycosylated; however, glycan structures vary widely between species. Invertebrate glycans are often 'foreign' to mammals, but many invertebrates are parasites which also immunomodulate the responses of the host, while invertebrate cell lines are potential 'cell factories' for production of secreted glycoprotein biopharmaceuticals.

Glycan arrays (i.e., glycans immobilized on glass slides in an array format) are proving important in understanding the interactions of glycans with various proteins. If we wish to investigate the innate or adaptive immune response of mammals to invertebrate glycoconjugates, a methodological hurdle is that most glycan arrays primarily focus on mammalian structures and not on those from other species. We are addressing this gap in the glycobiological toolbox by preparing arrays of either natural glycans from invertebrates or synthetic glycoconjugates displaying invertebrate glyco-epitopes.

To date we have printed (i) the neutral and anionic pools of N-glycans from honeybee royal jelly, (ii) the native and hydrofluoric acid-treated neutral N-glycan pools as well as fractionated N-glycans from the canine heartworm, (iii) mono- and disaccharides modified with zwitterionic phosphoethanolamine or phosphorylcholine and (iv) fucosylated and non-fucosylated forms of chitobiose and LacdiNAc, whereby (iii) and (iv) represent commonly-occurring glycan epitopes in non-vertebrate species.

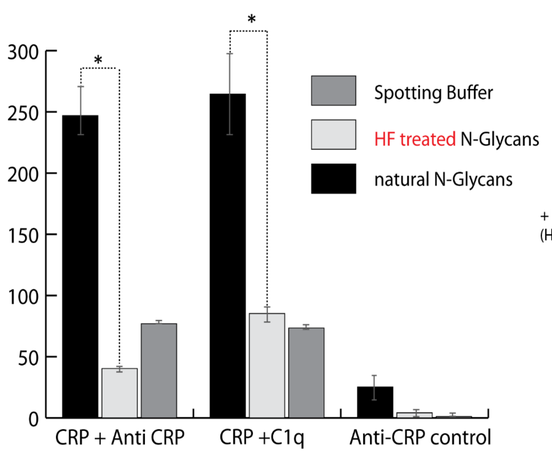
Glycophylogeny in animals

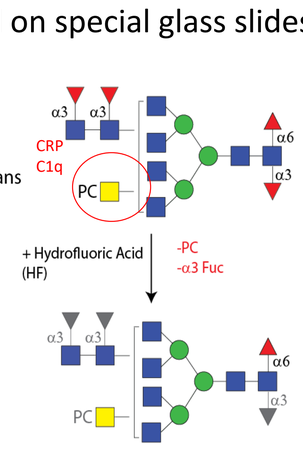


Examples of N-glycan structures from invertebrates using the SNFG colour code

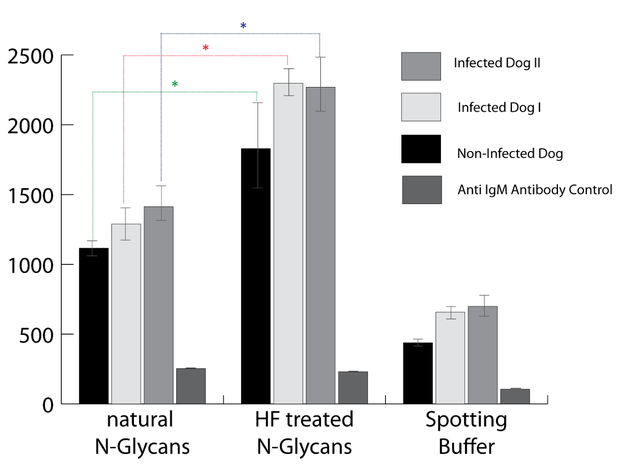
Binding of N-glycans from the canine heartworm

The canine heartworm (*Dirofilaria immitis*) is a mosquito-borne parasitic nematode with an expanding geographic range due to climate change. Adult worms were obtained after surgery of dogs and the N-glycans isolated and labelled with AEAB and immobilized on special glass slides.





Pre-treatment of N-glycans with HF to remove phosphoryl-choline and α3Fuc prevented binding to mammalian CRP (C-reactive protein, a pentraxin of the innate immune system).



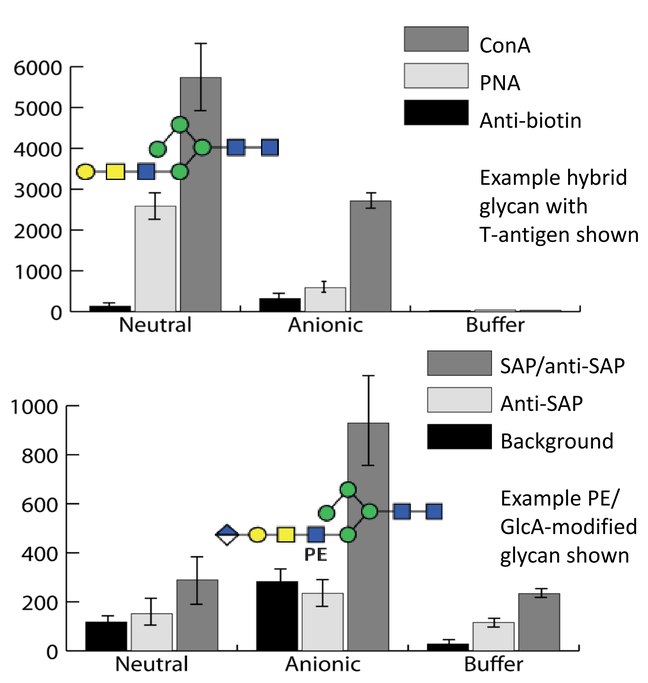
On the other hand, removal of phosphorylcholine and α3Fuc if anything increased the binding to heartworm glycans by IgM in sera of one non-infected and two infected dogs. Thus, the native heartworm glycans may aid that the parasite 'hides' from the adaptive immune system, so complicating vaccine development.

References:

1. Hykollari A, *et al.* (2018) Isomeric separation and pentraxin binding of anionic and zwitterionic N-glycans from royal jelly glycoproteins. *Mol Cell Proteomics* **17**:2177-2196
2. Martini F, *et al.* (2019) Highly modified and immunoactive N-glycans of the canine heartworm. *Nature Communications* **10**:*75*
3. Labrada K, *et al.* (2020) Zwitterionic phosphodiester-substituted neoglycoconjugates as ligands for antibodies and acute phase proteins. *ACS Chem Biol* **15**:*369–377*

Binding of N-glycans from honeybee royal jelly

Royal jelly is a product of glands of worker honeybees and is the sole food for queens; for centuries, it has also been used as a beauty or anti-aging product by humans. The question is whether there are positive or negative effects on the human immune system if exposed to royal jelly. Here, we isolated neutral and anionic N-glycan pools from royal jelly and immobilized them again using AEAB.

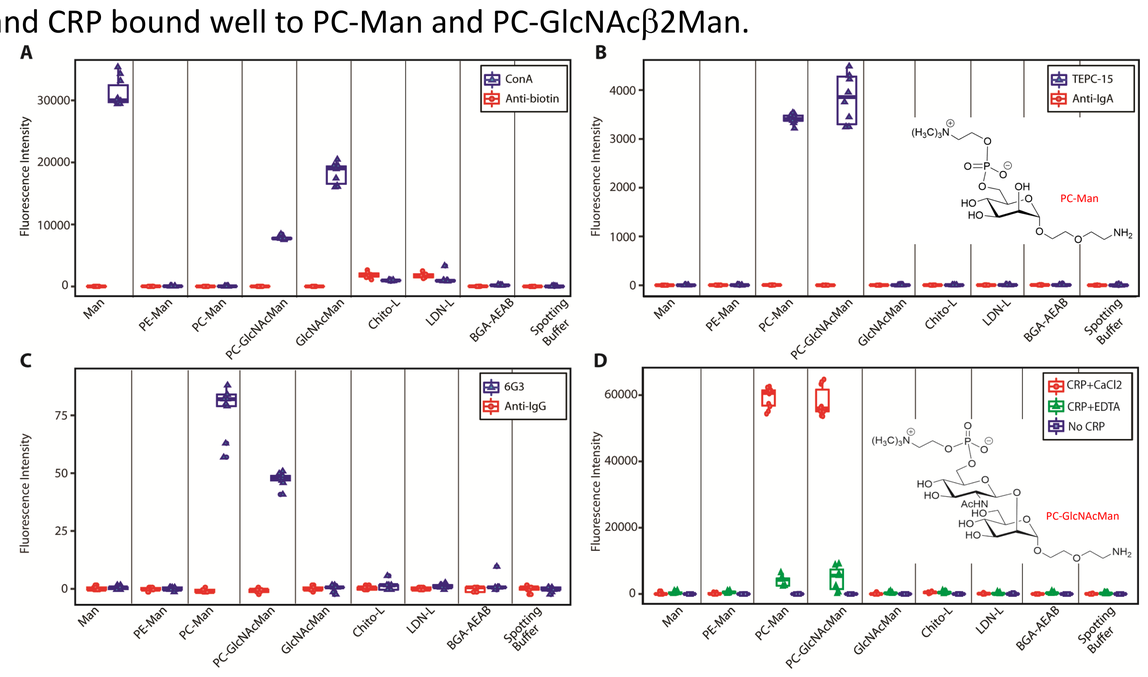


In first experiments, binding of plant lectins to royal jelly N-glycans was tested; the presence of oligomannosidic and T-antigen containing N-glycans correlates with concanavalin A (ConA) and peanut agglutinin (PNA) binding.

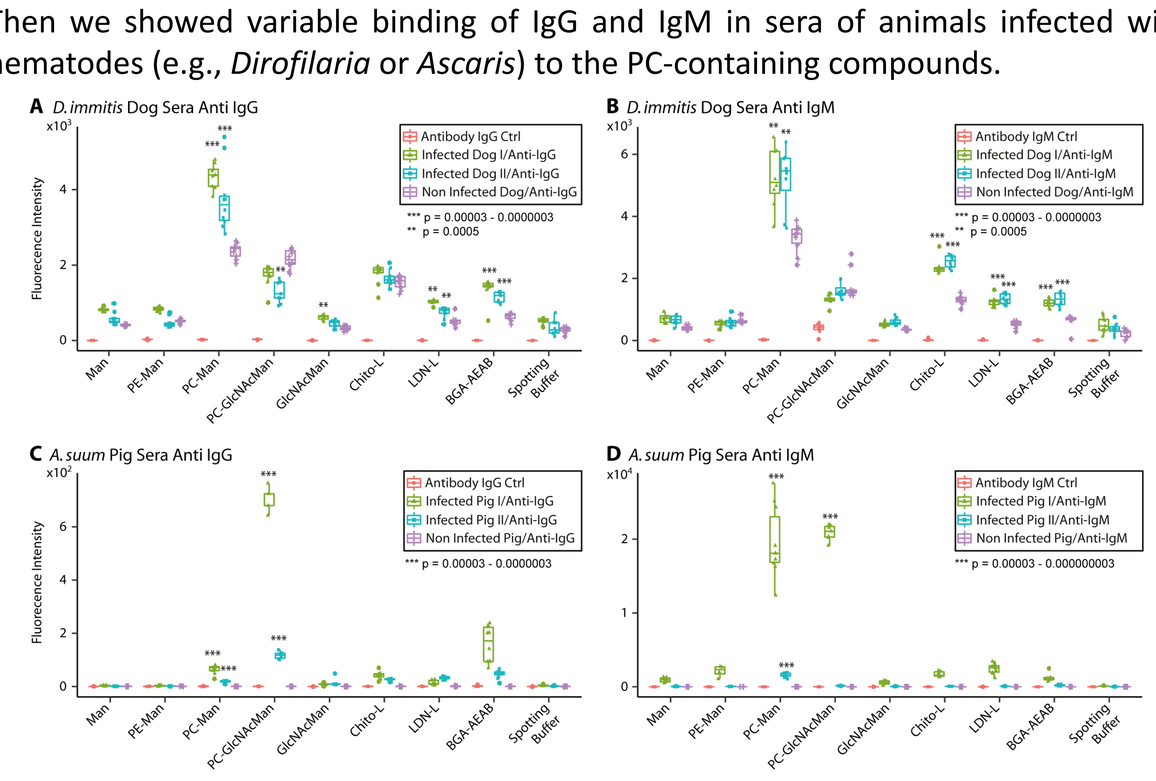
Then binding of the glycans to human serum amyloid P (SAP, a pentraxin binding phospho-ethanolamine or PE) was shown, especially for the anionic pool. This correlates well with the presence of PE on anionic N-glycans from royal jelly as proven by mass spectrometry.

Synthetic glycans in array format for testing specificity of lectins, antibodies and pentraxins

A series of simple mono- and di-saccharides (Man or GlcNAcβ2Man) with or without phosphorylcholine (PC) or phosphoethanolamine (PE) were synthesized on a 2-(2-azidoethoxy)ethyl spacer group; LacdiNAc (LDN) and chitobiose (chito) disaccharides were also printed on the same arrays prior to probing with ConA, two anti-PC antibodies (TEPC-15 and 6G3) or with C-reactive protein (CRP). The two antibodies and CRP bound well to PC-Man and PC-GlcNAcβ2Man.



Then we showed variable binding of IgG and IgM in sera of animals infected with nematodes (e.g., *Dirofilaria* or *Ascaris*) to the PC-containing compounds.



Summary: Development and use of invertebrate glycan arrays

We show that human serum amyloid P binds N-glycans derived from honeybee royal jelly (the food for honeybee queens, but also used as a beauty product), while human C-reactive protein (CRP) interacts with the phosphorylcholine-modified N-glycans of the canine heartworm (a mosquito-borne parasite).

Also, short phosphorylcholine-modified glycoconjugates are recognized not only by CRP, but by IgG and IgM in the sera of some nematode-infected animals. Thus our arrays are complementary to current resources and have high potential to yield new insights into glycan-mediated interactions with immune systems.