Glycoconjugate Journal (1996) **13:** 1–3

LETTER TO THE EDITOR

Glycobiology of *Plasmodium falciparum*: an emerging area of research

DANIEL C. HOESSLI[1], EUGENE A. DAVIDSON[2], RALPH T. SCHWARZ[3] and NASIR-UD-DIN[4]

[1]*Department of Pathology, Centre Medical Universitaire, University of Geneva, 1 Rue Michel-Servet, CH-1211 Geneva 4, Switzerland*
[2]*Department of Biochemistry and Molecular Biology, Georgetown University Medical School, Washington DC, 20007, USA*
[3]*Zentrum fur Hygiene und Medizinische Mikrobiologie, Philipps-Universitet Marburg, Robert-Koch-Strasse 17, D-35037 Marburg, Germany*
[4]*Institute of Biochemistry, University of Balochistan, Quetta, Pakistan*

Received 19 September 1995

Is there room for carbohydrates in malaria vaccine design?

Malaria is a dominant parasitic disease of man that causes more than 300 million clinical cases every year with an estimated mortality toll of between 1 and 3 million. Among the strategies elaborated to combat the disease, vaccines based upon asexual blood stage antigens are of special value as they mimic the natural immunity and may offer long-term protection. However, it has become apparent that the protection against human malaria achieved through vaccines currently tested in high endemicity areas is still insufficient [1] compared to that induced by efficacious vaccines against other infectious agents (e.g. smallpox, poliomyelitis) and, therefore, that improvements of existing malaria vaccines are necessary. At least one factor may be the role of covalently bound carbohydrate, present in the natural product but absent in recombinant material produced in bacteria.

Immunization with proteins purified from asexual blood stages of the human malarial parasite *P. falciparum* confers significantly more protective immunity in experimental animals than related recombinant proteins [2]. This has indeed been observed with many pathogenic parasites and suggests that the native proteins are better immunogens. The immunogenicity of malarial and other proteins is dependent on their proper proteolytic processing by host phagocytes and presentation as peptides bound to the cell-surface molecules of the major histocompatibility complex [3]. Such processing and presentation is governed not only by the proteolytic apparatus of the host phagocytes and the specificity of their peptide-binding surface proteins, but also by the conformation and post-translational modifications of the antigen [3].

The merozoite surface antigens MSP1 and MSP2 are of particular value for immunization because of their known immunogenic properties, their location at the surface of merozoites and involvement in binding of the merozoite to the host erythrocyte [4, 5]. Moreover, the presence of covalently bound saccharides [6–10] may affect both the conformation and antigenicity of the protein.

Protein glycosylation is now viewed as a highly dynamic and versatile set of post-translational modifications that regulate intermolecular and intercellular interactions as well as adaptive functions in cellular metabolism [11]. Evidence is accumulating to indicate that glycosylation-deficient cells may survive *in vitro*, whereas the same glycosylation defects introduced in the mouse blastocyst by homologous recombination result in embryonic death at mid-gestation [12, 13].

The presence of sugar residues in human malarial parasite proteins has been shown by metabolic labelling [14], lectin binding [15] and enzymatic digestion studies [16, 17]. A significant finding was the observation that proteins such as MSP1 may contain α-linked galactose residues which could influence the binding of the MSP1 to its antibody [16, 17]. The presence of naturally-occurring antibodies directed against the α-galactosyl epitope in humans and Old World monkeys [18], however, does not appear to perturb the blood-stage maturation of *P. falciparum*.

The lack of *N*-glycosylation in *P. falciparum* was suggested by Ramasamy and Reese [16] and confirmed recently [6–10]. Recent reports have indicated that some malaria proteins may contain O-linked saccharides [6–10]; similar linkages may be present in S. mansoni [19]. The presence and activity of an O-GlcNAc transferase was also suggested in *P. falciparum* [10]. O-GlcNAc modifications have been suggested to be regulatory [20], in that they may: (i) alter the conformation of a protein; (ii) regulate its level of serine/threonine phosphorylation; (iii) determine its involvement in multimeric complexes; and (iv) modulate its half-life and proteolytic processing in host macrophages.

Most O-GlcNAc modified-proteins described to date are cytoplasmic or nuclear [11]. However, lymphocyte activation has been shown to be accompanied by the appearance on cell-surface proteins of O-GlcNAc residues accessible to galactosyltransferase [21].

It should be noted that the predominant form of glycosylation in the malaria parasite is that associated with the GPI anchor structure, a component of many parasite surface proteins. MSP1 and MSP2 glycoproteins are anchored to the parasite membrane through glycosyl-phosphatidyl-inositol (GPI) structures [22] which have been shown to induce inflammatory cytokine production by host macrophages [23]. Among the malarial GPIs, two major structures have been identified [24]. One consists of an ethanolamine-phosphate-trimannose-glucosamine glycan linked to phosphatidylinositol [24], quite similar to the basic design of GPI anchors found in protozoan and metazoan glycoproteins and glycolipids [25]. The other contains four instead of three mannoses in its glycan moiety and represents the GPI structure that links glycoproteins to membranes in *P. falciparum* [24]. A modification of the inositol ring, making the GPI structure resistant to a phosphatidyl-inositol specific phospholipase C (PiPLC) was observed, but has not yet been characterized [24].

The 19 kDa, carboxy-terminal fragment of MSP1 bears the GPI anchor [26] and is involved in complex formation at the surface of the merozoite. Upon erythrocyte invasion, only the 19 kDa component of the complex is carried into the infected erythrocyte [26]. This specific behaviour may be related to the presence of the GPI-anchor on this fragment, as GPI anchors are known to transfer from one membrane to another [27, 28]. The anchor glycans and phosphatidylinositol termini also contribute to the pathogenic properties of the malaria toxins and represent antigenic targets *in vivo*. The concept of an 'anti-disease' vaccine was elaborated [29] to emphasize this aspect and develop immunological tools to inactivate these toxins. Finally, a protease activity also appears to be released from merozoites by a phosphatidylinositol-specific phospholipase C, suggesting the presence of a GPI-anchor on the p76 serine protease of *P. falciparum* [30].

Comprehensive investigations on the structure and functions of the different carbohydrate moieties in these asexual blood stage antigens are essential to understand the pathogenic properties of these molecules and to define their contribution to protective immunity. The peptides or proteins obtained by chemical synthesis or recombinant DNA technology that lack carbohydrate moieties on the protein surface or in the anchor are therefore devoid of important structural determinants of their *in vivo* toxicity, pathogenicity and immunogenicity.

Concluding remarks

In asexual stages of the parasite, a number of proteins appear to be glycosylated. The glycosylation pattern may vary depending upon the time of the asexual cycle during which this modification occurs. The specific modification sites [6–10] are unknown as is the complete structure of oligosaccharide chains in the diverse GPI anchors.

MSP1 and MSP2 glycoproteins belong to an ever increasing category of parasite GPI-anchored proteins. Several possible functions of these proteins have been considered and recognized [20]. It is possible that the further addition of O-linked GlcNAc in MSPs may regulate proteolytic processing and complex formation of MSP1 that occurs upon erythrocyte invasion by merozoites [26].

The GPI-anchor of MSP1, MSP2 and the p76 protease is likely to confer specific properties on merozoite and erythrocyte plasma membranes during invasion. The lateral mobility of GPI-anchored glycoproteins differs from that of transmembrane glycoproteins [31] and GPI-anchored surface molecules are also capable of transferring to other cells [27, 28]. The leishmanial GPI-molecules are known, for instance, to cause profound perturbations in the intracellular membrane traffic of their macrophage host [32], and it is conceivable that malarial GPI-molecules are in part responsible for the important plasma membrane remodelling that takes place during erythrocyte invasion [33].

Although recombinant parasite proteins expressed in eukaryotic hosts may be glycosylated, such modifications

are likely to reflect the properties of the expressing cell rather than those of the parasite itself. Since the parasite cannot be grown in quantity in the laboratory, a better understanding of its natural glycosylation is an important facet of future vaccine design.

References

1. Alonso PL, Smith T, Armstrong JRM, Schellenberg H, Masanja H, Mwankusye S, Urassa H, Bastos de Azevedo I, Chongela J, Kobero S, Menendez C, Hurt N, Thomas MC, Lyimo E, Weiss NA, Hayes R, Kitua AY, Lopez MC, Kilama WL, Teuscher T, Tanner M (1994) *Lancet* **344**: 1175–81.
2. Etlinger HM, Caspers P, Matile H, Schoenfeld H-J, Stueber D, Takacs B (1991) *Infect Immun* **59**: 3498–503.
3. Neefjies JJ, Momburg F (1993) *Current Opin Immunol* **5**: 27–34.
4. Gratzer WB, Dluzewski AR (1993) *Seminars Hematol* **30**: 232–47.
5. Su S, Sanadi AS, Ifon E, Davidson EA (1993) *J Immunol* **151**: 2309–17.
6. Dayal-Drager R, Hoessli DC, Decrind C, del Guidice G, Lambert P-H, Nasir-ud-Din (1991) *Carbohydr Res* c5–c8.
7. Nasir-ud-Din, Dayal-Drager R, Decrind C, Hoessli DC, Qazi MH, del Guidice G, Lambert P-H (1990) *J Chem Soc Pak* **12**:, 344–50.
8. Nasir-ud-Din, Dayal-Drager R, Decrind C, Bei-Hong-Hu, del Guidice G, Hoessli DC (1992) *Biochem Internat* **27**: 55–64.
9. Dieckmann-Schuppert A, Bender S, Odenthal-Schnittler M, Bause E, Schwarz RT (1992) *Eur J Biochem* **205**: 815–25. Nasir-ud-Din, Hassan M, Qazi MH, Fayyaz-ud-Din, Senaldi G, Hoessli DC, Walker-Nasir E (1992) *Biochem Soc Trans* **20**: 388.
10. Dieckmann-Schuppert A, Bause E, Schwarz RT (1993) *Eur J Biochem* **216**: 779–88.
11. Hart GW (1992) *Current Opin Cell Biol* **4**: 1017–23.
12. Ioffe E, Stanley P (1994) *Proc Natl Acad Sci USA* **91**: 728–32.
13. Metzler M, Gertz A, Sarkar M, Schachter H, Schrader JW, Marth JD (1994) *EMBO J* **13**: 2056–65.
14. Fenton B, Clark JT, Wilson CF, McBride JS, Walliker D (1989) *Mol Biochem Parasitol* **34**: 79–86.
15. Ramasamy R (1987) *Immunol Cell Biol* **65**: 419–424.
16. Ramasamy R, Reese RT (1986) *Mol Biochem Parasitol* **19**: 91–101.
17. Jakobsen PH, Theander TG, Jensen JB, Molbak K, Jepsen S (1987) *J Clin Microbiol* **25**: 2075–79.
18. Galili U (1993) *Immunol Today* **14**: 480–82.
19. Nyame K, Cummings RD, Damian RT (1987) *J Biol Chem* **262**: 7990–95.
20. Hayes BK, Hart GW (1994) *Current Opin Struct Biol* **4**: 692–6.
21. Torres C-R, Hart GW (1984) *J Biol Chem* **259**: 3308–17.
22. Haldar K, Ferguson MA, Cross GAM (1985) *J Biol Chem* **260**: 4969–74.
23. Schofield L, Hackett F (1993) *J Exp Med* **177**: 145–53.
24. Gerold P, Dieckmann-Schuppert A, Schwarz RT (1994) *J Biol Chem* **269**: 2597–606.
25. McConville MJ, Ferguson MAJ (1993) *Biochem J* **294**: 305–24.
26. Blackman MJ, Heidrich HG, Donachie S, McBride JS, Holder AA (1990) *J Exp Med* **172**: 379–82.
27. Rifkin MR, Landsberger FR (1990) *Proc Natl Acad Sci* **87**: 801–5.
28. Ilangumaran S, Arni S, Poincelet M, Theler J-M, Brennan PJ, Nasir-ud-Din, Hoessli DC (1995) *J Immunol*, in press.
29. Playfair JHL, Taverne J, Bate CAW, de Souza JB (1990) *Immunol Today* **11**: 25–27.
30. Braun-Breton C, Rosenberry TL, Pereira de Silva L (1988) *Nature* **336**: 457–59.
31. Zhang F, Crise B, Su B, Hou Y, Rose JK, Bothwell A, Jacobson K (1991) *J Cell Biol* **115**: 75–84.
32. Winter G, Fuchs M, McConville MJ, Stierhof YD, Overath P (1994) *J Cell Sci* **107**: 2471–77.
33. Simoes AP, Roelofsen B, Op den Kamp JAF (1992) *Parasitology Today* **8**: 18–21.