Surface expression of the conserved ribosomal protein P0 on parasite and other cells

Subhash Singh, Alfica Sehgal, Sanjeev Waghmare, Tirtha Chakraborty, Arunava Goswami 1, Shobhona Sharma *

Department of Biological Sciences, Tata Institute of Fundamental Research, Homi Bhabha Road, Mumbai 400 005, India

Received 9 June 2001; received in revised form 6 September 2001; accepted 12 September 2001

Keywords: Ribosomal phosphoprotein P0; Mammalian cells; Toxoplasma; Yeast; Surface expression

Invasion-blocking antibodies against the ribosomal phosphoprotein P0 of the human malarial parasite Plasmodium falciparum (PfP0) have been identified through a differential immunoscreen using immune and patient sera from malaria endemic regions of Eastern India [1]. Purified IgG from rabbit sera, raised against different domains of the PfP0 protein, show no effect on the intraerythrocytic stages of Plasmodium falciparum in culture, but inhibit the invasion of erythrocytes by merozoites in a concentration dependent manner [2,3]. The same antibodies against the PfP0 protein protect mice against challenge with a virulent strain of Plasmodium yoelii [4]. We have recently demonstrated this protein to be present on the surface of merozoites and gametocytes of Plasmodium [3,4]. The phosphoprotein P0 is highly conserved across eukaryotic organisms [5], and is related to the family of phosphoproteins P1 and P2, due to the highly homologous carboxy-terminal 22 amino acid domain [6]. The ribosomal function of P0 is mediated through a pentameric P1-P0-P2 complex, which forms the stalk of the large ribosomal subunit at the GTPase center [7].

P0 interacts with the elongation factor eEF2 [8], and is essential for the ribosomal activity and cell viability in yeast [9]. Through deletion analysis of the P0 protein, the ribosomal function has been mapped to amino acid position 185–230 in yeast P0 [10]. Since P0 is a very conserved protein [5], we wondered whether the surface expression of P0 is an exclusive property of Plasmodium cells, or if it is a general property of the P0 protein in other organisms as well. The presence of this protein on the host cell surface will have serious implications with the usage of this protein in a malaria vaccine. This question also has a direct bearing on the mechanism of translocation of this protein to the surface, as to whether it is a unique phenomenon for Plasmodium. Therefore, we decided to investigate the presence of this protein on different cell types. In this paper we report the localization of P0 protein on the surface of another Apicomplexan parasite Toxoplasma, yeast and mammalian cell lines using mono-specific cross-reactive anti-PfP0 antibodies.

In order to test for cross-reactivity of anti-PfP0 sera with different cell types, Western Blot analysis was carried out with total protein extract of these cell types. Anti-PfP0 antisera were raised in rabbits against recombinant GST-fusion proteins, PfP0N (amino acid 17–61) and PfP0C (amino acid 61–316), and purified as described earlier [3]. Specific single band reactivities of 38, 33, 34 and 38 kD proteins were observed with Toxoplasma gondii, yeast (Saccharomyces cerevisiae, strain EG103), Chinese Hamster Ovary (CHO) cells and human leukocyte protein preparations respectively, using
both anti-PfP0N and anti-PfP0C antibodies (Fig. 1). These molecular weights are the expected molecular weights of the respective P0 proteins. Since both anti-PfP0N and anti-PfP0C sera identify the same single protein band in each of the organisms tested, these results show that the sera are specifically recognizing the homologous P0 proteins.

*Toxoplasma gondii* tachyzoites, yeast spheroplasts, CHO cells, the human cell lines K562 (an erythroleukemic cell line), U937 (a lymphoma cell line), Daudi (a Burkitt lymphoma cell line) and SCC (an epithelial cell line), and peripheral human blood cells were subjected to solution immunofluorescence assay (SIFA). *Toxoplasma* tachyzoites were harvested by filtering the infected HFF cell lysate through a 3 μm filter and used for SIFA [11]. Yeast cells were grown with 1% glucose and harvested at an OD₆₀₀ value of 1–2 (~10⁵ cells). Yeast spheroplasts were prepared by treatment with zymolyase (Seikagaku Corp., Japan) [12], and used for SIFA. CHO cells were subcultured in 35 mm² Petri plates and the adherent CHO cells were used for SIFA assay after 24 h of growth. A distinct surface staining was observed with every tachyzoite of *Toxoplasma*, very similar to that shown by the surface marker SAG1 (gift from David Roos) (Fig. 2A). Yeast spheroplasts also showed distinct surface reactivity with anti-PfP0 sera samples, but the number of cells that reacted were 30–50% of the total cell population (Fig. 2B). With CHO cells, the number of cells found to be reactive was about 2–10% (Fig. 2C). In some of the reactive CHO cells, the surface reactivity could be detected at the membrane extensions quite distinctly (Fig. 2C, right panel). Human peripheral blood cells showed no SIFA activity (Fig. 2D). Approximately 100,000 total blood cells were scanned for each antibody tested, but no reactivity was detected with either red cells or leukocytes. Red cells do not possess any P0 protein, as fixed red cells do not show any IFA reactivity [2]. To test the leukocytes specifically, 10⁵ cells of the Buffy coat samples were collected from three donors and each sample was tested with the antibodies, and no surface reactivity was detected in any of these samples (data not shown). Each of these donors was healthy local (area of low malaria incidence) individual. Control monoclonal antibodies against glycophorin A (Pharmingen, USA) and HLA Class I (gift from S. Chiplunkar) reacted as expected with red cells and leukocytes (Fig. 2D). However, even though peripheral blood cells did not show any surface reactivity, hemopoietic cell lines K562, U937 and Daudi cells showed distinct cell staining (Fig. 2E). The epithelial cell line SCC also showed surface staining (Fig. 2E, d). The frequency of cells staining for these cell lines was low, varied with cell lines, and with different experiments, but was always within 2–5%. For each cell type, SIFA experiments were performed at least three times. Preimmune sera did not show any fluorescence activity with any of these different cell types used. The numbers and the patterns of reactivity were found to be similar irrespective of whether anti-PfP0N or anti-PfP0C serum sample was used.

The PfP0N and PfP0C possess no overlapping amino acid sequence. We have shown earlier that the anti-PfP0N and anti-PfP0C antibodies do not cross-react with each other [3]. On Western Blots both these sera light up specifically only the homologous P0 protein. Since both anti-PfP0N and anti-PfP0C antisera react to the cell surfaces with a similar pattern, it is unlikely that the surface reactivity recorded is due to non-specific P0-like cross-reactive domains located on the surface of the parasite.

About 10–15% of patients suffering from an autoimmune disorder, Systemic Lupus Erythematosus (SLE), possess autoantibodies against the conserved carboxy-terminal 22 amino acids of the P-proteins [13]. SLE serum sample has been used for immunoprecipitation study of *P. falciparum* protein extract, and distinct cross-reactivity with PfP0 has been observed [14]. It has been reported earlier that SLE sera do not react to the amino terminal domain of the P-proteins [13]. We have also recently reported that 10% of SLE patient sera samples cross-react with *P. falciparum* P0 protein, but only with PfP0C and not PfP0N protein [15]. We have shown that these PfP0C positive SLE sera samples inhibit the growth of *P. falciparum* through specific reactivity with PfP0C [15]. These results confirm the conserved structures of PfP0 and human P0.

So far only one report documents the surface localization of human P0-like protein, and this has been reported on the plasma membrane of human HepG2 and neuronal cell lines using purified SLE sera [16]. Results presented in this paper show that the surface expression of P0 is
regulated. While every *Plasmodium* [3] and *Toxoplasma* cell showed surface staining, the frequency for surface reactivity was 30–50% for yeast cells, and as low as 2–10% in mammalian cell lines. Peripheral blood cells (red cells and leukocytes) did not show any surface reactivity. The reason for this variation in SIFA reactivity is unclear. The low frequency of positively reacting cells is unlikely to be dead cells in the case of leukocytes, CHO, K562 and Daudi cell lines as we routinely performed the Trypan Blue exclusion assay to assess cell viability. We have also performed fixed sample analysis of these cell types, and all cells show the same patterns of reactivity with P0 and other markers such as beta-tubulin and ER-marker. Whether the SIFA reactive cells belong to an early stage of apoptosis remains to be tested.

From the cross-reactivity of anti-PiP0 antibodies it is clear that large protein domains of PiP0 are not suitable for a malaria vaccine. For vaccination purposes, smaller stretches of PiP0 with no cross-reactivity with the human P0 protein need to be identified. Passive immunizations with purified IgG obtained from immune adults, however, have been performed earlier on malaria patients with no apparent ill effects [17,18]. The experimental mice, which recovered completely in our passive immunization experiments, were healthy with no pathological symptoms of SLE [4]. The observation that peripheral blood cells do not show surface expression of P0 protein is an encouraging observation for passive immunotherapy. However, other human tissues also need to be examined for surface PiP0 expression. A large number (87%) of the malaria immune adults from Eastern India possessed anti-PiP0 antibodies, but did not exhibit any symptoms of SLE as documented through extensive questionnaire [1]. Indeed, it has been noted earlier that the proportion of SLE patients in malaria endemic areas is lower than that in areas with no malaria [19].
The biology of the P0 protein is complex and intriguing. It is apparent that it plays an essential role in the ribosomes [7,8] and also exhibits apurinic/apyrimidinic endonuclease activity in the nucleus [20]. Unlike most other ribosomal proteins, the level of P0 mRNA and protein are regulated, as has been shown in carcinogenic and apoptotic cells [21,22]. In flies, P0 also plays regulatory roles [23,24]. The ribosomal P2 protein homologue, L12, of *Neisseria gonorrhoeae* has recently been shown to play a role in gonococcal invasion of human endometrial Hec1B cells [25]. How P0 protein gets transported on the surface remains a question, as the deduced P0 protein sequences do not possess any consensus signal sequence or transmembrane motif. It is possible that the P0 protein is secreted out in a complex with other protein(s). Future studies centered on cell biological analysis of the membrane transportation of P0, along with attempts to map the various functions of P0 on sub-domains of the protein, will help in understanding the diverse functions of this protein.

Acknowledgements

We are indebted to David Roos (University of Pennsylvania, Philadelphia, PA) for antibodies to SAG1 antigen, and for the methodology with *Toxoplasma*. We also thank Shubha Chiplunkar (Cancer Research Institute, Mumbai) for providing us with the anti-HLA antibodies and the human cell lines and Satyajit Mayor (National Centre for Biological Sciences, Bangalore) for the CHO cells.

References


