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Short Communication

Anemia amelioration by lactose infusion during trypanosomosis could be associated with erythrocytes membrane de-galactosylation

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ABSTRACT

African trypanosomosis is a potentially fatal disease that is caused by extracellular parasitic protists known as African trypanosomes. These parasites inhabit the blood stream of their mammalian hosts and produce a number of pathological features, amongst which is anemia. Etiology of the anemia has been partly attributed to an autoimmunity-like mediated erythrophagocytosis of de-sialylated red blood cells (dsRBCs) by macrophages. Lactose infusion to infected animals has proven effective at delaying progression of the anemia. However, the mechanism of this anemia prevention is yet to be well characterized. Here, the hypothesis of a likely induced further modification of the dsRBCs was investigated. RBC membrane galactose (RBC m-GAL) and packed cell volume (PCV) were measured during the course of experimental trypanosomosis in mice infected with *Trypanosoma congolense* (stb 212). Intriguingly, while the membrane galactose on the RBCs of infected and lactose-treated mice (group D) decreased as a function of parasitemia, that of the lactose-untreated infected group (group C) remained relatively constant, as was recorded for the uninfected lactose-treated control (group B) animals. At the peak of infection, the respective cumulative percent decrease in PCV and membrane galactose were 30 and 185 for group D, and 84 and 13 for group C. From this observed inverse relationship between RBCs membrane galactose and PCV, it is logical to rationalize that the delay of anemia progression during trypanosomosis produced by lactose might have resulted from an induction of galactose depletion from dsRBCs, thereby preventing their recognition by the macrophages.

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1. Introduction

Trypanosomes are blood parasites and the causative agents of protozoan diseases known as nagana in animals, sleeping sickness and Chagas' disease in humans. In addition to *Trypanosoma brucei*, other causes of nagana include *Trypanosoma congolense* and *Trypanosoma vivax*, which are all transmitted to the mammalian host by a parasite-carrying tsetse during its blood meal (Jamonneau et al., 2004; Krafsur, 2009). Sleeping sickness and nagana are a major health problem in sub-Saharan Africa, and the economic impact of the animal disease is a severe constraint against the development of the region (Fenwick et al., 2005).

Anemia has long been established as a prominent pathological feature during trypanosomosis (Woodruff et al., 1973; Jennings et al., 1974; Salgado et al., 2011). It is largely attributed to the rapid loss of red blood cells (RBCs) from circulation, rather than a defective erythropoiesis (Biryomumaisho and Katunguka-Rwakishaya, 2007). A number of hypotheses have been put forward to explain the precise mechanism of this increased erythrocytes loss/destruction. They include: immuno-destruction, resulting from adsorption of parasites' antigenic material on to RBCs membranes (Woo and Kobayashi, 1975); membrane-lipid peroxidation, leading to oxidative damage of the RBCs (Igbokwe et al., 1996; Wolkmer et al., 2009); and phagocytosis of de-sialylated RBCs (Esievo et al., 1982). The last mechanism is the best-understood and widely accepted cause of the anemia (Guegan et al., 2013).

The erythrocyte membrane of human and animal species is heavily sialylated (Yaari, 1969; Mehdi et al., 2012), with the sialic acids (SAs) occupying terminal positions and masking an underlying galactosyl residue of cell membrane sialoglycoproteins (Varki, 2001). Membrane SAs have been shown to be a critical anti-recognition molecule, protecting RBCs from phagocytosis (Lee et al., 1988; Bratosin et al., 1998) by cells of the mononuclear phagocyte system (MPS). Removal of SAs usually by actions of sialidases results in exposing the previously masked penultimate galactosyl residue on RBC membrane, which becomes recognizable by the galactose specific lectins on macrophages, leading to erythrophagocytosis/sequestration of the asialo-RBCs, hence, the anemia (Bratosin et al., 1998; Guegan et al., 2013). Sialidases have been reported in different species of trypanosomes; their hydrolytic capacity on RBC membrane SAs, and the role in anemia pathogenesis are well studied (Nok and Balogun, 2003; Nok et al., 2003; Buratai et al., 2006; Coustou et al., 2012). As a result, the use of sialidase inhibitors has been thought to hold great prospect for chemotherapy or at least anemia prevention during trypanosomosis (Antoine-Moussiaux et al., 2009). However, there is yet to be any successful effort toward treatment of trypanosome-infected animals with sialidase inhibitors. The only effort in this direction is focused on the use of lactose infusion to ameliorate the anemia.

Although, the amelioration of anemia by lactose infusion during trypanosomosis is documented (Umar et al., 1998; Fatihu et al., 2008), there is a dearth of information on this, and the mechanism of the slow anemia progression remains inconclusive. To date, the only suggested mechanism is a competition for the galactose-specific lectins on MPS by lactose. However, due to the abundance of RBCs with exposed galactose during the disease as a result of the high parasite sialidase activity, it is logical to suppose that competition alone, between infused lactose and the circulating asialo-RBCs cannot account for such magnitude and efficiency of RBCs' protection from MPS. This has led to a speculation for the existence of additional mechanism(s) of anemia prevention by lactose infusion during trypanosomosis. In the present study, the presence of a potential glycoconjugate-modifying enzyme during lactose infusion to trypanosome-infected mice was examined. We previously reported an elevated serum glycosidase activity in the lactose-infused animals (Nok and Balogun, 2003), and herein, for the first time discuss its possible role in further modification of desialylated RBCs as a possible mechanism of asialo-RBCs' protection from recognition and sequestration by the MPS.

2. Materials and methods

Same-colony 5–6 weeks old female BALB/c mice, weighing 20–22 g, were procured from the animal unit of Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria-Nigeria. They were maintained on commercial pellet diet and water *ad libitum* under standard housing conditions. All animals used in this work were treated in accordance with the approved guidelines for animal experiments as reviewed by Ahmadu Bello University animal care and use committee. The animals were randomly separated to four groups (A–D) of four mice each. Starting from day 0; group A were not infused and not infected, group B were infused but not infected, group C were not infused but infected, and group D were infused and infected. Infection was by a single intraperitoneal inoculation with 10^4 *T. congolense* (stb 212) cells, while infusion was twice-a-day intraperitoneal administration of lactose in phosphate buffered saline (PBS) solution at a dosage of 0.5 g per kg body weight throughout the animal survival period.

Parasitemia was monitored daily by microscopic examination of thin blood smears, and in order to evaluate the loss of RBC that is often associated with the infection, microhematocrit method was used to monitor packed cell volume (PCV) at two days interval. To examine the relationship between PCV profiles and RBC surface modifications in trypanosome-infected animals and to investigate if the modifications were affected by lactose infusion, RBC ghosts were prepared as described in Natala et al. (2013), and analyzed. We have previously reported a correlation between parasitemia and trypanosome sialidase activity with RBC desialylation and decreased PCV in *T. congolense*-infected animals (Nok and Balogun, 2003). To investigate the possible further modifications occurring on RBCs during infection, susceptibility of the exposed galactosyl residues on membranes of de-sialylated RBCs to the released glycosidase was evaluated. A modified method of Stibler and Sydow (1983) was used to quantify RBC membrane galactose. Briefly, protein concentration of homogenous suspension of prepared RBC membranes of all mice groups (A–D) was determined according to Lowry