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Perspectives

Sialyltransferase activity probably counteracts that of sialidase as one of the possible mechanisms of natural recovery or stabilization of erythrocyte mass in trypanosome-infected animals - A perspective

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Attempts to unravel the mechanisms of development of anaemia in trypanosome-infected animals yielded varying reports with those that implicate trypanosomal sialidase (SD) being among those that enjoy prominence. Significant cleavage of sialic acid (SA) on erythrocyte membranes and terminal positions of erythropoietin (Epo) by SD, which is released during trypanosome infection of animals, results in their rapid clearance and destruction by cells of the mononuclear phagocytic system and in addition, in the case of Epo, decreased biologic activity of the hormone with consequent development of anaemia. Observations on free serum and erythrocyte surface SA profiles of trypanosome-infected animals suggest possible involvement of sialyltransferase (ST) in recovery or stabilization of red blood cell mass, which seems to be a probable explanation as to why some trypanosome-infected animals either recover from the anaemia or run a chronic infection with very low but stable erythrocyte mass. The speculated roles of ST open up a new and, probably, promising area of research; especially, if identification of mechanisms of natural resistance to trypanosome infection is of prime consideration in this contemporary period of research in molecular medicine.

Key words: Sialyltransferase, sialidase, sialic acid, erythropoietin, trypanosomosis, anaemia.

INTRODUCTION

Anaemia, manifested by low values of erythrocyte count, packed cell volume and haemoglobin concentration, has for long been identified as one of the principal features and major causes of death in the acute phase of trypanosomosis in livestock (Murray and Dexter, 1988; Esievo and Saror, 1991; Logan-Henfrey et al., 1992; Luckins, 1992; Buzza et al., 1995; Anosa et al., 1997; Nok and Balogun, 2003; Faye et al., 2005). Ever since the importance of this clinical feature of the trypanosomosis came into limelight, scientists ventured into research activities aimed at elucidating the mechanisms of its development in affected animals. Consequently, numerous and varying reports emerged (Igbokwe, 1989). The role of trypanosomal sialidase (SD) in the development of anaemia particularly in acute phase of trypanosomosis is unarguably one of the most documented of such reports.

SDs could possibly be contributing to the development of anaemia on two fronts; firstly, desialylation of the membranes of erythrocytes by the enzyme demasks galactose residues. The physicochemical alterations on the erythrocytes surface render them prone to destruction by cells of the mononuclear phagocytic system (Esievo et al., 1982; Muller et al., 1983; Bratosin et al., 1998; Umar et al., 1998; Nok and Balogun, 2003; Taylor and Authie, 2004; Ibrahim et al., 2005; Fatihu et al., 2008ab; Umar et al., 2008). Secondly, desialylation of erythropoietin (Epo)

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Abbreviations: SD, Sialidase; **SA**, sialic acid; **ST**, sialyltransferase; **Epo**, erythropoietin.

has been reported to result in enhanced *in vitro* and decreased *in vivo* biologic activities with concurrent reduction in plasma half-life of the hormone. How some animals in the trypanosusceptible group surmount anaemia during trypanosomosis remains an enigma. However, it is probable that sialyltransferase (ST) might be playing a role by regenerating sialic acid (SA) on the erythrocytes (Esievo et al., 1982; Kelm et al., 1986) and, perhaps, Epo thereby increasing their lifespan and plasma half-life, respectively.

It is pertinent to note that a good understanding of mechanisms of natural resistance to disease could, to a great extent, help in identification of molecular targets that could be exploited in design of therapeutic approach in this contemporary period of molecular medicine; just as identification of molecular and biochemical differences between parasitic pathogens and their hosts is pivotal to development of novel chemotherapies for parasitic infections (Chaudhuri et al., 2006). It is on this premise that we decided to review the functions of SAs and Epo, the role of SDs in the development of anaemia during the acute phase of trypanosomosis and how ST activity could, possibly, be playing a contributory role in mitigating the anaemia associated with the disease and, probably, constituting one of the key factors in conferring animals with resistance to trypanosome infection.

ANAEMIA IN TRYPANOSOME-INFECTED ANIMALS

Anaemia is simply defined as the deficiency of erythrocytes or haemoglobin concentration per unit volume of blood (Goossens et al., 1998). It is the most important feature of clinical trypanosomosis in both man and domestic animals (Luckins, 1992; Fatihu et al., 2003; Ogunsanmi and Taiwo, 2004). Indeed, anaemia has been implicated in the development of most tissue degenerative changes and, consequently, the disorders reported in trypanosome-infected animals (Logan-Henfrey et al., 1992). Reports have indicated that the onset of the anaemia is related to the appearance of trypanosomes in the blood and the severity associated with the level and initial peaks of parasitaemia (Dargie et al., 1979; Esievo et al., 1982; Sekoni et al., 1990; Nok and Balogun, 2003). Several and varying reports on the mechanisms of development of anaemia abound. For example, the roles of haemolytic factors such as haemolysin and free fatty acids (Tizard and Holmes, 1976; Tizard et al., 1977), immunologic mechanisms (Esievo and Saror, 1991; Logan-Hengrey et al., 1992), haemodilution (Fiennes, 1954; Maxie and Valli, 1978) (though strongly contested; Dargie et al., 1979a, 1979b), coagulation disorders (Boreham and Facer, 1974; Valli et al., 1978a), depression of erythrogenesis (Igbokwe, 1989) and release of trypanosomal SDs have been implicated in the development of anaemia in trypanosomosis.

The diverse nature of the reports notwithstanding, the general consensus is that the anaemia in both human and animal trypanosomoses is predominantly the result of haemolytic crisis, in which the erythrocytes are being destroyed by an expanded mononuclear phagocytic system (Igbokwe and Mohammed, 1991; Buzza et al., 1995; Umar et al., 1998: Nok and Balogun, 2003; Taylor and Authie, 2004). Following infection with the trypanosomes, an intense erythrophagocytosis, which is mainly extravascular, due to the activated and expanded mononuclear phagocytic system is elicited, where the half-life of the erythrocytes is significantly reduced (Witola and Lovelace, 2001; Taylor and Authie, 2004).

About SAs

SAs are negatively charged molecules existing on various glycan types and are generated by a family of 18 ST genes differently expressed among tissues (Kaufmann et al., 1999; Ellies et al., 2002; Borman, 2004). SAs are most commonly linked to the penultimate galactose or *N*-acetylgalactosamine on glycan branches and can be essential in the formation of ligands for endogenous SA specific-specific lectins such as the siglecs, the selectins or pathogen receptors. The majority of SAs attached to plasma components are α2-3 linked to and produced by up to six ST3Gal genes encoding sialyltraansferasesST3GAL, I-VI. SAs are amongst the most important molecules of life. They are found occupying the terminal positions on macromolecules and cell membranes, thus, playing roles in many biological and pathological phenomena (Schauer, 2000a). Indeed, the fact that SAs occupy external positions on glycoproteins and gangliosides, as well as on the outer cell membranes, underscores their significance in cell biology (Schauer, 1982; Varki, 1992; Kelm and Schauer, 1997; Schauer and Kamerling, 1997; Traving and Schauer, 1998; Wang, 2005). The biosynthesis of these molecules may act as a coding system, since they are able to interact with high specificity and selectivity with carbohydrate-binding proteins including lectins, antibodies, receptors and enzymes (Thomas, 1996). SAs have a dual role; not only are they indispensable for the protection to and adaptation to life, but they have been found to be utilized by many pathogenic microorganisms. It has been documented that the terminal SA of glycans is an important residue in affecting cell behaviour (Schauer, 2000a). Usually, sialyl residues are linked to the inner sugar residue, galactose via α2,3-, α2,6- or α2,8-linkage or linked to galactosamine or *N*-acetyl galactosamine via α2,6-linkage. Moreover, SA can also be linked to the C8 position of another SA residue. The biosynthesis of these molecules may act as a coding system, since they are able to interact with high specificity and selectivity with carbohydrate-binding proteins including lectins, antibodies, receptors and enzymes (Thomas, 1996; Wang, 2005).

The roles of SA in physiological and pathophysiological processes

The roles being played by SAs have been divided into more general ones, irrespective of their variable structures, and those exerted by the chemical modifications. The biology of SA may be viewed from its dual role either in masking recognition sites (Schauer, 1985) or, in contrast, representing a biological target, whereby it allows recognition by a receptor protein and, thus, serving as ligand or a counter receptor (Kelm and Schauer, 1997). The latter role of SAs is known to be modified or even abolished by SA subtituents, most effectively by *O*-acetyl groups. Hence, the multifaceted role of SAs makes these molecules very difficult to understand, as the environment of these monosaccharides and the nature of the molecule to which they are attached may influence the biological effects (Schauer, 2000a). By virtue of their negative charge, SAs are involved in the binding and transport of positively charged molecules as well as in the attraction and repulsion of cells and molecules (Faillard and Schauer, 1972; Schauer, 1982; Varki, 1992; Kelm and Schauer, 1997; Schauer and Kamerling, 1997; Schauer, 2000b). SAs, as components of glycoproteins, are responsible for the high viscosity of mucin lining and protecting endothelia, for instance, in the intestine. They influence the conformation, for example, of gangliosides (Siebert et al., 1996) and contribute to the supramolecular structures in cell membranes, thus, influencing their functions. Recognized hydrophobic subtituents of SAs such as *O*-acetyl, *O*-methyl or hydroxylation with *N*-acetyl group, providing more hydrophilic properties and even a further site for glycosidic linkage, may influence the physicochemical properties of SAs (Schauer, 2000a).

The anti-recognition effect of SAs is exerted by their negative charge in combination with the bulky hydrophilic molecule. This involves masking of the penultimate sugar which nature has designated to be recognized by receptors, like galactose, or the less specific shielding of antigenic sites in macromolecules or in cell membranes (Kelm and Schauer, 1997; Schauer, 1985). SAs, thus, render cells as “self”, not allowing recognition by the immune system. The loss of SA makes these cells more “no-self” and, therefore, vulnerable (Schauer and Kamerling, 1997; Kelm and Schauer, 1997; Schauer et al., 1999; Schauer, 2000a). SA has repeatedly been implicated in the metastatic process (Yogeeswaran and Salk, 1981). It has been reported that inhibition of sialylation and *O*-glycan extension and sialylation reduces the metastatic potential of cancer cells (Bresalier et al., 1991; Kijima-Suda et al., 1986). Indeed, evidence shows that altered ST expression, with resultant increase in tissue sialylation, has a significant correlation with oncogenesis, tumour progression and lymph node metastases (Wang, 2005). It is possible that chains carrying SA may regulate the interaction of cancer cells with other cells and with the cell matrix. These chains may therefore be responsible for adhesion as well as anti-adhesion, and for extending the survival time of cancer cells in the blood stream (Wang, 2005). SA may also be involved in growth regulation (Carraway et al., 1992).

Desialylation leads to, in the first case, recognition by galactose-specific lectins and in the second case, to better recognition of macromolecules and cells by the complement or the immune system. The first effect targets molecules and cells to specific sites, often, leading to their degradation and may, thus, be of physiological or pathological importance. Reported example of this phenomenon was the uptake of desialylated glycoproteins, for instance Epo, by hepatocytes (Ashwell and Morell, 1974; Wang, 2005). A similar function was observed between liver and spleen macrophages and erythrocytes, first observed with human erythrocytes (Jancik and Schauer, 1974), which have lost 10 - 20% of their surface SA (1 - 2 × 10^7 molecules/cell) either by ageing or under the influence of SD from blood serum or microorganisms.

Erythrocytes bind to phagocytes via their demasked galactose residues to a galactose-specific receptor and ultimately are taken up and degraded (Muller et al., 1983). This mechanism can work without the involvement of immunoglobulin or the complement and represents the main sequestration process of aged cells (Bratosin et al., 1998). SD-treated lymphocytes also attach to macrophages by this mechanism; however, they are not engulfed but are released after about one-day incubation due to resialylation of the cell surface (Fischer et al., 1991).

SDs and anaemia in animal trypanosomosis

SDs are enzymes of SA catabolism. These enzymes have been reported to occur in *Trypanosoma vivax*, *Trypanosoma congolense*, *Trypanosoma brucei*, *Trypanosoma evansi*, *Trypanosoma rhodesiense*, *Trypanosoma rangeli* and *Trypanosoma cruzi* (Esievo, 1979; Reuter et al., 1987; Engstler et al., 1991, 1992; Engstler and Schauer, 1993; Nok and Uemura, 1998; Nok and Balogun, 2003; Nok et al., 2003; Buratai et al., 2004; Umar et al., 2008). SDs may be contributing to the development of anaemia on two fronts. Firstly, desialylation of erythrocyte membranes demasks galactose residues via which such erythrocytes binds to phagocytes through a galactose-specific receptor and is ultimately taken up and degraded (Esievo et al., 1982, 1986; Muller et al., 1983; Bratosin et al., 1998; Umar et al., 1998; Nok and Balogun, 2003; Taylor and Authie, 2004; Umar et al., 2008). Secondly, desialylation of Epo, a glycoprotein containing terminal SA and the hormone that is responsible for the regulation of erythropoiesis in mammals and for the recovery of erythrocyte mass following haemorrhage and haemolytic crisis, has been reported to result in reduced biologic activity of the hormone, *in vitro*, and its rapid uptake by hepatocytes (Ashwell and Morell,

1974; Sticher et al., 1988; Wang, 2005). Trypanosomal SDs' cleavage of SA on erythrocytes surfaces and glycoproteins, thus, results in their reduced lifespan and plasma half-lives, respectively. Consequently, they are rapidly cleared from the blood, which results in anaemia. The result of the combined effects of SD on erythrocytes and Epo during the acute phase of the trypanosomosis, among other factors, is therefore an accelerated development of anaemia.

Erythrophagocytosis and SD activity

It appears that cleavage of SA on erythrocyte membrane is the initial requirement for erythrophagocytosis to ensue (Muller et al., 1983) as it enhances the rate and strength of binding of desialylated erythrocytes to galactose-specific lectins on surfaces of macrophages (Kelm et al., 1986; Umar et al., 2008). Significant cleavage of erythrocyte membrane SA was found to concur with high parasitaemia levels and, possibly, period of highest release of SD from trypanosomes (Esievo et al., 1982; Nok and Balogun, 2003, Umar et al., 2003, 2008). Also, it has been observed that the period of decline in erythrocyte mass and therefore development of anaemia coincides exactly with the time of significant loss of erythrocyte membrane SA that is sequel to the activity of trypanosomal SD (Esievo et al., 1982; Nok and Balogun, 2003; Umar et al., 2003, 2008). Desialylation of erythrocytes is subsequently followed by their sequestration and phagocytosis by macrophages (Ibrahim et al., 2005).

Indeed, erythrophagocytosis requires the initial rate-limiting step in which desialylated erythrocytes bind to β-galactose-specific lectins on macrophages. This step is then accompanied by the phagocytic step that involves serum complement substances (Kuster and Schauer, 1981; Muller et al., 1983). The finding that the rate-limiting binding step of desialylated erythrocytes to macrophages is inhibited both *in vitro* and *in vivo* by galactose and β-glycosidically bound β-D-galactosyl residues (Schlepper-Schafer et al., 1980; Muller et al., 1981; Nok and Balogun, 2003; Ibrahim et al., 2005; Umar et al., 2008) is sufficient evidence to suppose that desialylation of erythrocytes is the predisposing factor to erythrocyte destruction, and subsequent development of anaemia, by cells of the mononuclear phagocytic system.

About Epo

Erythropoiesis (erythrocyte production) is the process whereby a fraction of primitive multipotent haemopoietic stem cells becomes committed to red cell lineage, forming first burst forming units-erythroid, then colony forming units-erythroid, normoblasts, erythroblasts, reticulocytes and ultimately, the mature erythrocytes (Hoffbrand and Pettit, 1993; Moritz et al., 1997). Epo is the principal factor in the regulation of erythropoiesis. It is a glycoprotein hormone that contains terminal SA and is responsible for the regulation of erythropoiesis in mammals and for the recovery of erythrocyte mass following haemorrhage and haemolytic crisis. Although other factors may synergize with Epo, mice that are null mutants for either Epo gene or its receptor die about day 13 of development, thus, demonstrating the critical importance of the hormone. Epo, like protein hormones, acts by binding to a specific Epo receptor (EpoR) embedded in the plasma membrane of target cells, the red cell precursors in the bone marrow (Wikipedia, 2006).

The concept that erythrocyte production was regulated by a hormone was first proposed in 1906 by Carnot and Deflandre (Jelkmann, 1994). Since then, it took nearly half a century for the existence of such a factor, named Epo, to be conclusively proven. It took another 20 years before the hormone could be purified in amounts sufficient to deduce its amino acid structure and 10 years later the world was presented with the gene for Epo (Moritz et al., 1997). The gene encoding human Epo was cloned in 1985, which led to the production of recombinant human Epo (Faruki and Kiss, 1995). A dose of recombinant Epo developed for veterinary use lasts for a day but the effects are observed 5 days later when the cell proliferation it has induced is mature enough for release into circulation. Today, it is known that Epo is a heavily glycosylated protein with a molecular weight of about 30,000 Daltons. A considerable interspecies cross-reactivity for Epo has been reported (Konishi et al., 1993; Wen et al., 1993). This is because of the existence of a close amino acid identity between human Epo and that of other mammalian species (Lin et al., 1986; Jelkmann, 1992; Wen et al., 1993; Marti et al., 1996). Indeed Sheep has been widely used as a developmental animal model because it is suitable for the study of erythropoiesis, haemoglobin switching and Epo production as the sites of erythropoiesis and the type of haemoglobin produced at different stages; most closely resemble the situation in humans (Wintour et al., 1991).

Epo and other growth factors in erythropoiesis

Epo is primarily produced by the cells of the peritubular capillary endothelium of the kidney and is responsible for the regulation of erythrocyte production (Tabbara, 1993; Faruki and kiss, 1995; Moritz et al., 1997). In exercising this role, the Epo acts in concert with other growth factors such as stem cell factor (SCF), IGF-1 and IL-3 to stimulate the proliferation and maturation of responsive bone erythroid precursor cells (Jelkmann, 1992). Epo has been found to interrupt the normal apoptotic cycle experienced by the erythroid progenitors as they progress from CFU-E through the basophilic erythrocyte stage (Longmore et al., 1993; Koury and Bondurant, 1992). Concomitant with this decrease in apoptosis, factors such as SCF will then expand the progenitor population by stimulating their proliferation, while IGF-1

promotes new red cell release by directing erythroid maturation through the orthochromic erythroblast stage (Koury and Bondurant, 1992). In conjunction with IL-3, Epo also seems to have effect on the earliest erythroid precursor, the BFU-E (which gives rise to CFU-E).

Other functions of Epo

In addition, recent works indicated that Epo can promote the survival of septal cholinergic neurons *in vivo*, suggesting a possible role of Epo in central nervous system (Koury and Bondurant, 1992). Reports on *in vitro* studies have also suggested that the hormone may play a role in thrombocytopoiesis by (1) inducing both megakaryocyte DNA synthesis and cytoplasmic process formation, and (2) synergizing with thrombopoietin to stimulate the proliferation of Coliform-Forming-Unit megakaryocyte (Moritz et al., 1997). Lower levels of Epo are synthesized in the liver, and some evidence suggests that there is an additional contribution from macrophages in the bone marrow (Jelkmann, 1992). Astrocytes have also been proposed to be a source of Epo in the central nervous system (Masuda et al., 1994). Human Epo is a polypeptide consisting of 165 amino acids, containing one or O-linked and 3 N-linked carbohydrate chains (Sawyer et al., 1989).

The stimulus to Epo production and the determinants of its plasma levels

Ninety percent (90%) of Epo is produced in the peritubular cells of the adult kidney in response to a decrease in tissue oxygenation (Jacobson et al., 1957; Koury et al., 1988). There is evidence indicating that the protein in these cells which detects oxygen saturation in blood is a haeme-containing moiety (Goldberg et al., 1988). As the oxygen partial pressure (PO_2) of the plasma, a function of the haematocrit decreases, Epo concentration will increase (Erslev et al., 1980). There are also observations suggesting that, normally, there is an inverse correlation between serum Epo levels and erythrocyte mass (Spivak, 1986). Serum Epo levels are dependent on the rate of production and the rate of clearance of the protein (Imai et al., 1990).

Quantitation of serum Epo serves as a diagnostic adjunct in determining the cause of anaemia or erythrocytosis. Aplastic anaemia, thalassemias, megaloblastic anaemia, myelodysplastic syndromes, haemolytic anaemia and anaemia due to iron deficiency all result serum Epo elevation (Erslev, 1991; Abdelrahman et al., 1996; Huang et al., 1997). Whereas, Epo levels in patients with secondary anaemia due to renal failure, end-stage renal disease, anaemia of chronic disorders (chronic infections and rheumatoid arthritis) autoimmune haemolytic diseases, Acquired Immune Deficiency Syndrome (AIDS) and malignancy are generally inappropriately low for the degree of anaemia (Abdelrahman et al., 1996; Harris and Winkelmann, 1996). This is most likely caused by an impaired ability of the diseased kidney to produce adequate quantities of the Epo (Erslev, 1991). The low to normal serum Epo levels in many of these conditions have also been attributed to generation of IL-1 and a factor that that has been shown to be inhibitor of Epo activity. Polycythaemia rubra vera or primary erythrocytosis (an increase of erythrocyte mass) results from unstimulated over production of erythrocytes. Hence, the increase in haemoglobin causes decreased production of Epo, which results in subnormal serum levels of the hormone (Garcia et al., 1982). Secondary polycythaemia, which is also characterized by an increase in total erythrocyte mass, occurs as a physiological response to elevated levels of circulating Epo caused by tissue hypoxia. The hypoxia may be due to such factors as pulmonary fibrosis, cardiovascular disease, prolonged exposure to high altitude, and abnormal forms of haemoglobin or drug treatment (Wild, 1994). Some tumours produce Epo and in these cases, the hormone may be used as a tumour marker to monitor the effectiveness of treatment.

Effects of desialylation on Epo structure and its biological activity

The circulating form of human Epo is a 165 amino acid glycoprotein with molecular weight of approximately 30000 (Jacobs et al., 1985; Lin et al., 1986). Although the cDNA for the hormone predicts a molecule with 166 amino acid residues, the carboxy-terminal arginine is removed in a post-translational modification (Moritz et al., 1997). Three potential sites for *N*-linked glycosylation, which are all filled, have been identified. One *O*-linked carbohydrate moiety is also present. The effects of glycosylation are complex. For example, unglycosylated *E. coli*-derived Epo shows full biological activity *in vitro*, but apparently requires glycosylation for full activity *in vivo*. Thus, *E. coli*-produced and deglycosylated, naturally derived Epo show very low activity in animal studies (Krantz, 1991). Consistent with these findings, various glycosylation patterns show variable effects. For example, desialylated Epo exhibits both enhanced *in vitro* and decreased *in vivo* activity, an effect attributed to changes in structural conformation of the hormone and exposure of galactose residues, which are recognized, bound and cleared by Kupffer cells (Ashwell and Morell, 1974; Goldwasser et al., 1974; Sticher et al., 1988; Wen et al., 1993; Wang, 2005). The branching pattern of fully sialylated Epo also makes a difference in biological activity. Predominantly tetra-antennary branched Epo shows activity equivalent to “standard” Epo, while predominantly bi-antennary branched Epo shows three-fold more activity *in vitro* but only 15% of normal activity *in vivo* (Jelkmann, 1994). Fairly recent studies indicate that only N-linked, and not O-linked sugars, are important

in Epo functioning (Higuchi et al., 1992).

Epo function in trypanosome-infected animals

Haemolytic anaemias are expected to trigger reticulocyte response via the stimulatory activity of Epo (Sanchez-Medal et al., 1969; Schalm et al., 1977; Moritz et al., 1997). However, most reports on investigations conducted on the bone marrow response to anaemia induced by trypanosome infection suggest the contrary. For example, *T. vivax* and *T. congolense* infections of ruminants failed to induce any significant reticulocyte response (Valli et al., 1978b; Anosa and Isoun, 1980; Valli and Mills, 1980; Anosa, 1988; Igbokwe, 1989) or induced very slight response Mackenzie and Cruickshank, 1973; Buzza et al., 1995). Bone response to anaemia caused by experimental *T. brucei* infection in goats was also reported to be poor (Igbokwe and Mohammed, 1991). However, few reports indicated that *T. brucei* infection of mice, rats and rabbits produced considerable reticulocyte response (Anosa et al., 1977; Jenkins et al., 1980; Anosa et al., 1988).

The mechanisms underlying the cause of ineffective erythropoiesis in trypanosome-infected animals have, at best, remained speculative. Anaemia of trypanosomosis appears to be similar to the anaemia of chronic disease in man in which ineffective erythropoiesis, retention of iron in the mononuclear phagocytic system and low Epo concentration have been observed (Dargie et al., 1979; Baer et al., 1987; Murray and Dexter, 1988). Buzza et al. (1995) suggested that the presence of inhibitory factors in trypanosome infection, which are not yet understood, might be responsible for the impaired reticulocyte response in affected animals. Although there have been only few reports on the effect of trypanosome infection on Epo concentration in animals (Buzza et al., 1995; Suliman et al., 1997), there are indications that trypanosome infections either cause reduced plasma Epo concentration or some changes in its structure which render the hormone ineffective in relation to its biological activity. For example, Igbokwe and Anosa (1989) observed that plasma of sheep with artificially induced anaemia elicited reticulocyte response in mice but not plasma of sheep with anaemia induced by *Trypanosoma vivax* infection, even though the two anaemias were of comparable degrees. This, finding either suggests complete lack or reduced Epo level in the plasma of the trypanosome-infected sheep or presence of ineffective Epo. In their report, Buzza et al. (1995) observed that even though there was an elevated Epo levels in *T. congolense*-infected calves, there was an ineffective erythroid response, thus, suggesting poor biological activity of the Epo. Moreover, desialylation of Epo was shown to result in enhanced *in vitro* and decreased *in vivo* activity of the hormone, an effect that was attributable to the exposure of galactose residues, which were recognized, bound and cleared by hepatocytes (Goldwasser et al., 1974; Sticher et al., 1988; Wen et al., 1993).

The occurrence and possible release during trypanosomosis of SDs by trypanosomes (Esievo, 1979; Reuter et al., 1987; Engstler et al., 1992; Nok and Uemura, 1998; Nok and Balogun, 2003; Buratai et al., 2004) could be detrimental to Epo function. Although the effect of trypanosomal SD activity on Epo has not been investigated, it may be reasonable to attribute the ineffective erythropoiesis observed in trypanosome-infected animals (Igbokwe, 1989; Igbokwe and Anosa, 1989; Buzza et al., 1995) to the activity of the enzyme produced by the parasites during infection, which desialylates the hormone thereby reducing both its biological activity and plasma half-life. Effect of SD activity on Epo concentration and function, thus, exacerbates the rapidly developing anaemia due to erythrocyte destruction by the cells of the mononuclear phagocytic system and may be a contributory factor to the reported dyserythropoiesis (Igbokwe, 1979; Igbokwe and Mohammed, 1991; Anosa et al., 1997; Logan-Henfrey et al., 1999) in trypanosome-infected animals.

About STs

ST is a family of enzymes that catalyzes the transfer of SAs to nascent oligosaccharides at terminal positions on cell surfaces, glycoproteins and glycolipids (Sticher et al., 1988; Bosshart and Berger, 1992; Hennet et al., 1998; Kaufmann et al., 1999; Borman, 2004; Ellies et al., 2002; Wang, 2005). However, STs were reported to have higher affinity for glycoproteins than for cells (Colowick and Kaplan, 1982). These enzymes are members of the glycosyltransferases family of which about 20 varieties are known in mammals (Sticher et al., 1988; Kiljanski et al., 1990; Bosshart and Berger, 1992; Hennet et al. 1998; Borman, 2004). Of recent, genes encoding STs have been found to be expressed in different mammalian tissues (Ellies et al., 2002; Borman, 2004) and, in human, the enzyme is now found to be elaborated at different levels in many tissues (Wang, 2005). About 18 ST genes have been found to be expressed by among tissues (Ellies et al., 2002; Grahn et al., 2002; Harduin-Lepers et al., 2005). The study of STs is relatively complicated (Harduin-Lepers et al., 2005; Teintenier-Lelievre et al., 2005) by the fact that all involve tumour-associated changes in the expression of cell-surface sialylglycoconjugates. Also, the specificity studies and cloning of STs from different cell types reveal significant differences between enzymes with similar activities

Each ST is specific for a particular sugar substrate. Hence, the 20 different STs can be distinguished on the basis of the acceptor structure on which they act and on the type of sugar linkage they form. For example, a group of STs add SA with an α2,3-linkage to galactose, while other STs add SA with an α2, 6-linkage to galactose or *N*-acetylgalactosamine. A peculiar type of STs add SA to

other SA units with a α2,8-linkage, forming structures referred to as polySA.

Classification of STs

STs (STs)) can be classified into four families according to the carbohydrate linkage they synthesize: the ST3Gal (α2,3-ST), ST6Gal (α2,6-ST), STGalNAc and ST8Sia (α2,8-ST) families. All enzymes of the ST3Gal family transfer Neu5Ac residues in α2,3-linkage to terminal Gal residues found in glycoproteins or glycolipids (Wang, 2005). The most abundant SA linkage found among mammalian cell surface oligosaccharides is of the α2,3 variety and can be produced independently by four STs that each, nonetheless, bear unique substrate preference among glycolipids, asparagines(N)-linked glycans and serine/ threonine (O)-linked glycans (Hennet et al., 1998). STs have also been found to be developmentally regulated and differentially expressed among various cell types. For example, expression of α2,8-linked SAs is much less common than α2,3-linkages and appears restricted to small subset of glycoproteins. α2,6-linkages are also less abundant than α2,3-linked forms and are generated by at least four distinct gene products.

Structure of STs

All STs have similar architecture. They are type II transmembrane glycoproteins that predominantly reside in the transGolgi compartment (Harduin-Lepers et al., 2005). The first glycosyltransferase structure was determined in 1994 (Borman, 2004). STs have a short N-terminal cytoplasmic tail, a unique transmembrane domain, and a stem region with a variable length from 20 to 200 amino acids followed by a large C-terminal catalytic domain. The vertebrate ST amino acid sequences described up to date show overall limited sequence identity (from 15 to 57% for human STs), but share four peptide conserved motifs called the sialylmotifs: L (large), S (small), motif III (Patel and Balaji, 2005) and motif VS (very small) (Jeanneau et al., 2004; Harduin-Lepers et al., 2001). These four motifs are common in all ST. In humans, STs are expressed in many tissues at different levels (Tsuji 1996; Kaufmann et al., 1999; Jeanneau et al., 2004.

ST activity and resistance to trypanosome infection in animals

The phenomenon of resistance to trypanosome infection in some animal species and how some animals surmount the anaemia induced by trypanosome infection have remained subjects of investigation. For example, it is well documented that in Africa certain breeds of cattle have the unique ability to exhibit tolerance to trypanosome infection (Esievo et al., 1986; Ogunsanmi et al., 2000; Naessens et al., 2003). Similarly, some breeds of sheep and goats and many species of wild life have shown increased resistance to trypanosome infections (Itard, 1989). Although the molecular and genetic basis of this so-called trypanotolerance in this group of animals is not well understood, it is known that the trait is manifested by the animals' greater capacity to control parasitaemia and anaemia during the infection (Esievo et al., 1986; Logan-Henfrey et al., 1999; Naessens et al., 2003). However, it is most intriguing that some animals in the trypano-susceptible group were found to recover from the anaemia over several months over several months of the infection with trypanosomes, while some were found to remain chronically infected with persistently low erythrocyte values (Anosa et al., 1997). Bone marrow response to anaemia (Igbokwe and Mohammed, 1991; Anosa et al., 1997; Logan-Henfrey et al., 1999) contributes little in the recovery of the red blood cell mass in this latter group because of trypanosome-induced dyserythropoiesis (Murray and Dexter, 1988; Igbokwe, 1989; Buzza et al., 1995). Therefore, how these trypanosusceptible animals occasionally surmount the anaemia by maintaining the low erythrocyte count at a fairly constant level remains largely an enigma. However, there are evidences to suppose that ST plays a role of resialylating the asialoerythroctes (Esievo et al., 1982; Kelm et al., 1986) and, probably, asialoEpo even though this was not investigated in any of the studies mentioned.

Although there is lack of information on the serum activity of ST during trypanosome infection in animals, it could be postulated that by regenerating SAs on the exposed galactose residues of red blood cells, ST prevents the desialylated red blood cells' binding to and subsequent uptake by cells of the mononuclear phagocytic system (for example, the Kupffer cells' lectin) and so prolongs the erythrocytes' life spans. In the same vein, regeneration of SA on desialylated Epo could increase its plasma half-life and biologic activity. Thus, ST activity in the regeneration of SA on desialylated erythrocyte membranes and Epo might be a contributory factor in the recovery or stabilization of erythrocyte mass in some of the trypanosome-infected animals.

CONCLUSION

It may be logical to surmise that SD and ST are key player enzymes in the pathogenesis of anaemia in trypanosome-infected animals with the former, elaborated by trypanosomes, cleaving off SA bound to erythrocytes' membrane and terminal positions of Epo to cause an accelerated decline in erythrocyte mass with subsequent development of anaemia and the latter regenerating the SA on the exposed galactose residues on erythrocyte surfaces and desialylated Epo, thereby prolonging the erythrocytes' lifespan as well as the plasma half-life and biologic activity of Epo. Although the possible roles of ST

remain at best speculative, they might be one of the mechanisms of natural resistance to trypanosome infection in animals, thus, obligating future research efforts to make this unexplored area a prime focus.

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