Lysis of African trypanosomes by human plasma lipoproteins

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The salivarian trypanosomes T. gambiae and T. rhodesiense are the causative agents of African sleeping sickness, whilst the closely related T. brucei is one of several that cause nagana in cattle. In man, T. brucei brucei is not normally infective and the haemoflagellate is lysed upon exposure to human plasma. Rifkin (1978a) has identified the trypanocidal factor present in human plasma as high-density lipoprotein (HDL), but neither the particular sub-class involved nor the mode of action has been defined (Rifkin, 1983). The present findings suggest that the smaller HDL particles are the lytic agents to T. brucei brucei, apparently because they are rich in apolipoprotein (apo) A.

A capillary stabitate of T. brucei brucei, derived from a clone of strain MA1G427 MI Tat 1.6 (kindly provided by Dr. R. Klein, M.R.C. Parasitology Unit, Molteno Institute, University of Cambridge, Cambridge, U.K.) and containing approx. 2 x 10^9 trypanosomes, was used to infect individual mice. Blood was collected 72 h later during the rising phase of parasitaemia and, after centrifugation, theuffy coat was resuspended in phosphate-buffered saline/glucose, pH 8.0, I = 0.109, and applied to a column of DEAE-cellulose (DE-52, Whatman) (Lanham & Godfrey, 1970). Trypanosomes were eluted free of other cells with the buffer, washed three times and their proteins labelled by incubation at 4°C washed three times and suspended in ice-cold minimum essential medium containing 10% (v/v) heat-inactivated foetal calf serum and 5 μCi of [4-5^-H]leucine/ml for 30 min at 37°C (Rifkin, 1978b). Cells were collected by centrifugation at 2°C, washed three times and suspended in ice-cold minimum essential medium containing 1% (w/v) defatted bovine serum albumin at 2 x 10^7 trypanosomes/ml. Human and rabbit HDL (p 1.063–1.21) were obtained by sequential, preparative ultracentrifugation of fresh plasma (Owen et al., 1984). In some experiments HDL was separated into apo A-rich (HDL-A) and apo E-rich (HDL-E) fractions by affinity chromatography on heparin-Sepharose (Weisgraber & Mahley, 1980), whilst in other studies tyrosine residues in apolipoproteins of HDL-A were converted to 3-nitrotyrosine with tetranitromethane (Brinton et al., 1986). Lipoproteins were dialysed against phosphate-buffered saline, pH 7.4 containing 10 mm-glucose and 0.3 mm-EDTA and their concentrations expressed in terms of their protein content (Lowry et al., 1951). Incubations of labelled trypanosomes and HDL were carried out for up to 2 h at 37°C and cell lysis assessed by measuring the release of acid-precipitable tritium into the culture medium as described in the legend to Fig. 1. This technique is known to correlate closely with direct estimates of lysis by microscopic examination (Rifkin, 1978b).

When ^3H-labelled trypanosomes were incubated with human HDL there was 6–20 times more acid-precipitable radioactivity released into the culture medium than with rabbit HDL or buffer (Fig. 1a). Fractionation of this human HDL established that the small, apo A-rich sub-class were the active cytotoxic particles; the large, cholesteryl-ester and apo E-rich molecules were essentially non-lytic (Fig. 1b). When apolipoproteins of HDL-A were chemically modified with tetraniromethane there was a marked decrease in their ability to lyse the trypanosomes (Fig. 1b), suggesting that the cytotoxic action of these HDL particles is mediated by their apolipoprotein constituents rather than by their lipid molecules. Further studies will be needed to establish which of the HDL-A apolipoproteins is involved and whether it acts at a specific site on the parasite surface.

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Fig. 1. Ability of human HDL to lyse T. brucei brucei as a function of time

Suspensions (0.5 ml) of ^3H-labelled T. brucei brucei (2 x 10^7 trypanosomes/ml) were mixed with 0.5 ml of HDL (1 mg of protein/ml, i.e. within the normal human physiological range) or buffer and incubated at 37°C. Aliquots (150 μl) were removed and, after rapid centrifugation to sediment the cells, the supernatant (100 μl) was mixed with 2 ml of ice-cold trichloroacetic acid (10%, w/v). The precipitates were washed once and dissolved in 1 ml of 0.1 M-NaOH for measurement of radioactivity by liquid scintillation counting. (a) •, human HDL; ■, rabbit HDL; ○, phosphate-buffered saline. (b) ●, human HDL-A; □, human HDL-E; O, human HDL-A modified with tetraniromethane. Each point represents the mean of duplicate determinations and the counts present at zero time (187–229 d.p.m.) have been subtracted at each time point.
**Altered steroid hormone and prostaglandin metabolism during chlamydial infection in sheep**

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Chlamydial infection is associated with premature labour in sheep, and causes necrosis of placental tissue. The effect of *Chlamydia* infection on the synthesis of progesterone was studied, by measuring serum progesterone in seven infected and seven control animals. Progesterone inhibits the release of prostaglandin (PG) E₂ and PGF₁α from the pregnant uterus (Taylor *et al.*, 1978). Local inflammation at the site of infection may also stimulate intrauterine PGE₂ synthesis (Thorburn & Challis, 1979).

The amniotic and allantoic sacs and the utero-ovarian vein were cannulated in seven *Chlamydia*-infected sheep and seven uninfected controls. Infection was by subcutaneous inoculation of 10³ infectious particles of an ovine abortion strain of *Chlamydia psittaci*. PGE₂ was detected by radioimmunoassay of uterine fluids (Leaver & Seawright, 1982) without prior extraction, as parallel binding curves were obtained in the presence and absence of fluid. However, PGE₁, in utero-ovarian plasma was immediately extracted, as an interfering factor, and PGE₁ degradation, were detected in plasma. Anti-PGE₁ antiserum was purchased from the Institut Pasteur, Paris, and anti-rabbit IgG was donated by the Scottish Antibody Production Unit.

The plasma progesterone of *Chlamydia*-infected sheep was not significantly different from control values until day 120 of gestation (see Fig. 1). The concentration of progesterone decreased significantly between day 119 and day 135 of gestation in *Chlamydia*-infected animals, but not in control, uninfected sheep. The decline in plasma progesterone in control sheep occurred between day 139 and 145 of pregnancy. Therefore, the decline in circulating progesterone was observed 20 days earlier in *Chlamydia*-infected sheep, and 16 days before delivery.

The concentration of PGE₁, in amniotic and allantoic fluids in control animals was low (2–4 ng/ml), between day 115 and day 128 of gestation. In contrast, an elevated concentration of PGE₁, and pulsatile release of this prostaglandin, was observed in *Chlamydia*-infected sheep from day 119, which gradually increased until delivery. The release of PGE₁ into the utero-ovarian vein just before parturition, observed in control animals, was impaired or inhibited in *Chlamydia*-infected sheep. The magnitude of the increase in PGE₁ observed in *Chlamydia*-infected amniotic fluid (over 2 ng/ml), suggested that the PGE₁ was of uterine, rather than leucocyte, origin. The relation of the timing of PGE₁ release, to the decline in plasma progesterone, also suggested an endocrine control of this PGE₁ release.

Abbreviation used: PG, prostaglandin.

In summary, changes in circulating steroid hormones, and intrauterine PGE₁, were detected in *Chlamydia*-infected sheep. These changes may precipitate the premature labour associated with *Chlamydia* infection.

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**Fig. 1. Plasma progesterone in Chlamydia-infected (a) and control (b) sheep during late pregnancy**

The mean date of lambing is indicated with an arrow. Progesterone was extracted from plasma using diethylether, and results were corrected for efficiency of solvent extraction (70 ± 6%), and detected using the antiserum of Scaramuzzi *et al.* (1974).