Transgenic mice expressing human apolipoprotein A-I have sera with modest trypanolytic activity in vitro but remain susceptible to infection by *Trypanosoma brucei brucei*

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Abstract Although *Trypanosoma brucei brucei* fatally infects livestock in much of sub-Saharan Africa, humans are innately resistant to infection, apparently because high-density lipoproteins (HDL) in human serum lyse this unicellular protozoan parasite. Recently, we demonstrated that purified human apolipoprotein (apo) A-I, the major protein (*M*, 28,016) constituent of HDL, had full trypanolytic activity in vitro whereas the apoA-I of cattle and sheep was non-lytic. In the present study, we have sought to confirm the trypanocidal capability of human apoA-I by studying four lines of transgenic mice expressing (supra)physiological serum levels of this polypeptide. Although trypanolysis in vitro by sera from transgenic mice (15.1 ± 1.3% [mean ± SEM], n = 30) was considerably less than by human sera (typically 60–80%), it was nevertheless significantly greater than by control sera (8.5 ± 1.1%, n = 10; $P < 0.001$) and correlated with the concentration of human apoA-I ($r = 0.56$, $P < 0.001$). When trypanosomes were incubated at 37°C with human serum or with human apoA-I for 30 min (i.e., within the pre-lytic period) they lost their ability to subsequently infect mice; trypanosomes incubated with transgenic mice serum remained infective. Furthermore, transgenic mice were fully susceptible to infection when inoculated with 10^3 trypanosomes; both the initial detection of trypanosomes in the blood (3–4 days) and the time to death (5–6 days) were no longer than control mice. This apparent paradox between the action of human apoA-I in human serum and in mouse serum was investigated further. Normal mouse serum, but not bovine or sheep serum, had a powerful antagonistic effect of the trypanolytic activity in vitro of human serum with about 50% inhibition when each serum was present at 25% (v/v). This inhibitory action was lost when mouse serum was depleted of its HDL by chemical precipitation, whilst mouse HDL isolated by ultracentrifugation was itself inhibitory. Homologous and heterologous reconstitution experiments, using as starting material HDL from which nonpolar lipids had first been removed, established that the antitrypanolytic agents in mouse HDL were not the phospholipid constituents, rather the apolipoprotein fraction was active. These findings provide further support for the trypanocidal capability of human apoA-I, but also suggest that the mouse is an inappropriate species in which to test the ability of the human apoA-I transgene to impart trypanocidal activity to the serum and to confer resistance to infection by *T. b. brucei*.—**Owen, J. S., M. P. T. Gillett, and T. E. Hughes.** Transgenic mice expressing human apolipoprotein A-I have sera with modest trypanolytic activity in vitro but remain susceptible to infection by *Trypanosoma brucei brucei*. *J. Lipid Res.* 1992. **33:** 1639–1646.

Supplementary key words high density lipoprotein • trypanolysis

The ability of African trypanosomes of the *brucei* subgroup to infect humans and cause disease appears to depend upon their sensitivity to the trypanolytic properties of normal human blood. Thus, human serum or plasma lyses *Trypanosoma brucei brucei* (1) and the parasite, although benignly present in a variety of African game animals and the cause of the fatal wasting disease, ngana, in domestic livestock, is ordinarily unable to infect man. By contrast, *T. b. gambiense* and *T. b. rhodesiense* the infective agents for chronic and acute forms, respectively, of human sleeping sickness, are relatively resistant to the cytolytic action of human serum. Whilst this difference has been widely used to detect potentially human-infective trypanosomes, on the basis of their continued infectivity towards laboratory rodents after incubation with normal human serum (2–4), the underlying mechanism at the molecular level is ill-defined. Central to such an understanding is the clear identification of an active trypanolytic constituent in human sera which, by analogy with the host range of

Abbreviations: apo, apolipoprotein; HDL, high-density lipoprotein; $_{pd}$HDL, partially delipidated high-density lipoprotein.

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T. b. brucei, must also be present in baboon sera, another nonpermissive host (5, 6), but absent or inactive in sera of livestock and laboratory animals.

Rifkin (6–8) was the first to report that the trypanocidal factor of human serum was high-density lipoproteins (HDL), inasmuch as HDL isolated by ultracentrifugation from man or baboon was cytotoxic whereas that from permissive hosts, rat and rabbit, was inactive. Although ultracentrifugation is known to induce alterations in HDL integrity and protein content (9, 10), other studies have confirmed her proposal that the trypanocidal action of human serum resides with native HDL particles (10, 11). However, circulating HDL are heterogeneous, multimolecular complexes; they can be fractionated according to density, size, charge, and apolipoprotein composition, and they have different metabolic properties (9, 12, 13). As yet, there is no consensus as to whether the trypanolytic properties of HDL are associated with all or only certain subclasses of HDL (9, 14, 15) or whether a particular apolipoprotein or lipid constituent, or combination of both are required (6, 14–16).

Apolipoprotein (apo) A-I, a polypeptide of 243 amino acids (M_r 28,016) of known sequence (17, 18), constitutes about 70% of the total apolipoproteins of human plasma HDL and analogous apoA-I molecules are major structural components of HDL isolated from other mammals (19, 20). Recently, we demonstrated that apoA-I purified from human or baboon HDL was trypanolytic, whereas apoA-I isolated from the non-lytic HDL of cattle or sheep was benign (15). Moreover, incorporation of human apoA-I into bovine or sheep HDL conferred trypanolytic activity on the particles; conversely, reducing the apoA-I content of human HDL was accompanied by loss of trypanocidal activity (15). In the present study, we have examined further this capability of human apoA-I for trypanolysis by studying transgenic mice expressing (supra)physiological levels of human apoA-I in their serum.

MATERIALS AND METHOD

Transgenic mice

Four lines of transgenic C57BL/6 mice, which had integrated the human apoA-I gene and had overexpressed human apoA-I in their livers, were established: line 19 (plasma levels of human apoA-I of ~100 mg/dl, compared to 80–140 mg/dl in normal human plasma), line 4 (~200 mg/dl), and lines 26 and 38 (~300 mg/dl). The construct used in the generation of the lines was a 13 kbp fragment encoding the entire human apoA-I gene and approximately 9.5 kbp of 5'-flanking sequence. Plasma concentrations of mouse apoA-I were ~150 mg/dl in control animals and in lines 19 and 4, but in line 26 and line 38 the levels were about 80–100 mg/dl indicating suppression of the mouse apoA-I. Fuller details of these human apoA-I transgenic mouse strains will be presented elsewhere (T. E. Hughes, M. E. Swanson, I. M. St. Denny, D. F. Nottage, D. S. France, J. R. Paterniti, Jr., and K. Burki, unpublished results).

Lipoprotein preparation

Blood from control or transgenic mice was obtained under terminal anesthesia from the axillary vessels, clotted on ice for 2 h, and serum was isolated by centrifugation for 5 min at 13,000 *g*. If required, preparation of mouse HDL was begun on the same day; otherwise aliquots of sera were stored at −20°C for up to 6 months for use in trypanocidal assays. Blood was collected from other species and the plasma was separated as described previously (15, 21). Bovine HDL, mouse HDL, human HDL (all d 1.063–1.21 g/ml) and human apoA-I were prepared by our standard methods (15, 21). Levels of human apoA-I in transgenic mouse sera were measured by a noncompetitive enzyme-linked immunoadsorbent assay (22) and in mouse HDL by commercial radial immunodiffusion plates (Immuno Ltd., Sevenoaks, Kent, U.K.). Removal of apoB-containing (i.e., non-HDL) lipoproteins and of total lipoproteins from plasma was accomplished by treatment with phosphotungstate and magnesium ions (10). Partially delipidated HDL ($_{pd}$HDL), in which the hydrophobic, neutral lipid (triglyceride and cholesteryl ester) core of the particles had been removed (23, 24), was prepared by first mixing 2 mg HDL-protein in 0.5 ml phosphate-buffered saline with 25 mg potato starch (Sigma Chemical Company, Poole, Dorset, U.K.) in 13 × 100 mm siliconized glass tubes. After rapid freezing in liquid nitrogen and lyophilization, the nonpolar lipids were extracted with ice-cold heptane (3 × 5 ml), and traces of residual solvent were evaporated under a gentle stream of N_2. The $_{pd}$HDL was then solubilized with 0.5 ml H_2O for 15 min at 37°C, separated from the starch by low-speed centrifugation, and sterilized by passage through a 0.22-μm filter (Spin-X; Costar (U.K.) Ltd., High Wycombe, Bucks). For reconstitution experiments, aliquots of $_{pd}$HDL (2 mg protein/ml from mice, humans, and cattle) were initially fully delipidated by extraction with methanol and diethyl ether (25) and the residual apo$_{pd}$HDL was dried. The organic solvent containing the polar lipids of $_{pd}$HDL, essentially phospholipid and cholesterol, was then evaporated to dryness and small unilamellar liposomes were prepared by sonication with 1.0 ml phosphate-buffered saline using a micro-probe (26). Recombination of dried 2-mg batches of apo$_{pd}$HDL with homologous or heterologous liposomes was achieved by rotary mixing at room temperature to produce clear, stable, discoidal complexes (15, 26).

Trypanosomes and trypanolytic assays

The trypanosomes used in all studies were derived from clone MIAG 427 (MITat 1.6) of *T. b. brucei* which produces an acute, monomorphic infection in laboratory mice. Suspensions of *T. b. brucei* were freshly prepared from the blood of 3-day infected T/O mice by the standard procedure described previously (10, 15) and were used immediately. The trypanolytic activity of transgenic mouse sera or of HDL preparations was assessed in two ways. First, by incubation with 2.5×10^7 trypanosomes/ml for 2 h at 37°C and subsequent estimation of the percentage lysis by phase-contrast microscopy (7, 10, 15). Second, by injecting normal mice intraperitoneally with trypanosomes (10^6) that had been pre-incubated with test sera or HDL for 30 min at 37°C (i.e., within the pre-lytic period (27)) and then monitoring their survival time. To establish whether transgenic mice were resistant to infection, 10^3 trypanosomes in 0.3 ml were injected intraperitoneally into mice; the initial appearance of trypanosomes in the blood of these mice was recorded (the prepatent period (28, 29), when typically the blood contained about 10^6 trypanosomes/ml), together with their survival time.

Statistics

Regression lines were calculated by the method of least squares and results are expressed as means ± SEM; statistical differences were determined by Student's two-tailed unpaired *t* test.

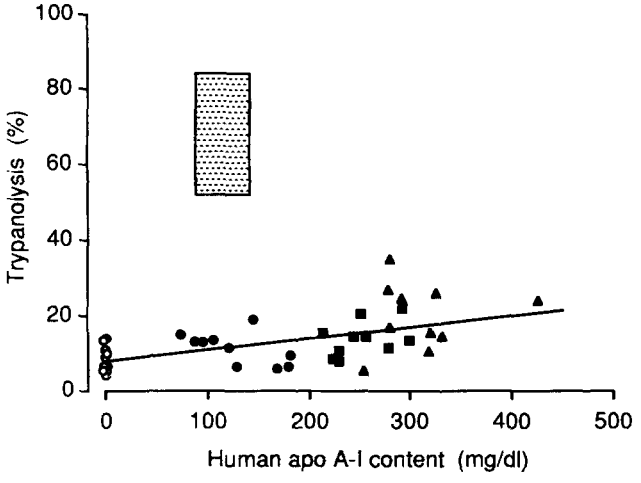

Fig. 1. Lysis in vitro of *T. b. brucei* by transgenic mouse serum as a function of serum concentration of human apoA-I. Suspensions of trypanosomes (2.5×10^7/ml final concentration) were incubated at 37°C for 2 h with 25% (v/v) sera from control C57BL/6 (○) mice or from lines 19 (●), 26 (■), and 38 (▲) of the transgenic mice. The shaded rectangle indicates the range for 18 plasma samples from healthy human individuals, using the identical assay for assessing trypanolytic activity (10).

TABLE 1. Effect of pre-incubation of *T. b. brucei* with transgenic mice sera on their infectivity towards normal mice

Pre-incubation Substance[a]	Survival Time of Mice (n = 4)
	days
Buffer only	3.9
Human plasma	>15[b]
Human apoA-I	>15[b]
Bovine apoA-I	6.1
Control C57BL/6 mouse serum	4.2
Transgenic mouse serum (line 26)	4.8

[a]Trypanosomes (10^6 cells in 0.1 ml) were incubated at 37°C for 30 min with 0.2 ml sera or 0.2 ml buffer with or without 0.25 mg of human or bovine apoA-I and then the mixture was injected intraperitoneally into normal T/O mice. The values are means (n = 4) for survival counted in increments of 0.5 days from the time of injection.

[b]All mice were still alive after 15 days and were considered free from infection.

RESULTS

Trypanolytic activity in vitro of transgenic mouse serum

Our standard assay of 25% (v/v) serum and 2 h incubation at 37°C was used, which gives a mean lysis of 76 ± 8% (n = 18, range 52–84%; **Fig. 1**) for human plasma (10). Although the mean trypanolytic activity of transgenic mice sera (15.1 ± 1.3%, n = 30) was only moderately greater than that of control sera (8.5 ± 1.1%, n = 10; $P < 0.001$), half the samples had activity greater than the control range (4.6–13.8%) and there was a good correlation with the concentration of human apoA-I in the serum ($r = 0.56$, $P < 0.001$; Fig. 1). These findings were consistent with a preliminary experiment using sera that had been stored for several months at −20°C and had been thawed two or more times: the mean percentage lysis of sera from 14 transgenic mice (12.9%) was twice as high as that of 6 control samples (6.4%) and similarly was significantly correlated with the content of human apoA-I ($r = 0.48$, $P < 0.05$). Several of the most lytic transgenic sera were monitored by phase-contrast microscopy throughout the incubation period. As with human serum, there was a lag period of about 45 min before lysis was apparent and also the typical intermediate cell-swelling stage was evident during conversion of the slender form to cell ghost (30) (data not shown).

When trypanosomes were incubated for 15 min at 37°C with human serum or with purified human apoA-I and then injected into normal T/O mice, all animals were alive at 15 days indicating complete loss of infectivity (**Table 1**). By contrast, trypanosomes incubated with transgenic mice sera showed little loss of subsequent infectivity towards mice.

Inhibitory effects of mouse serum on trypanolysis in vitro

Because the trypanolytic activity of transgenic mouse sera was only modest compared to human plasma, despite its two- or threefold greater content of human apoA-I, several experiments were performed to establish whether mouse sera might contain inhibitory factors. Addition of increasing amounts of control or transgenic mouse serum to a suspension of *T. b. brucei* during incubation at 37°C for 2 h with 25% (v/v) human plasma, steadily decreased the trypanolytic effect of the human plasma (**Fig. 2A**). By contrast, bovine or sheep plasma had no effect. Similarly, when increasing concentrations of purified human apoA-I were co-incubated with a constant amount (25%, v/v) of normal mouse plasma, their cytolytic effect was substantially impaired (Fig. 2B). Such inhibitory effects of mouse serum persisted after removal of all its apoB-containing lipoproteins (i.e., low-density and very-low-density lipoproteins) by precipitation, but not when the HDL fraction was also removed (Fig. 2C). This apparent antagonistic action of mouse HDL on the lytic activity of human plasma was confirmed by additional experiments using mouse HDL isolated by preparative ultracentrifugation (data not shown).

To assess whether the anti-trypanocidal action of mouse HDL might be due to the lipid constituents, we attempted to reconstitute the apolipoproteins of human HDL (apoHDL) with the total lipid fraction of mouse HDL (and also, as controls, with the lipids of bovine HDL and human HDL) using co-sonication (26, 31). In our hands, these experiments were largely unsuccessful; the solutions remained turbid and tended to separate into two phases, presumably because of the presence of appreciable quantities of hydrophobic triglyceride and cholesteryl ester (26, 31). As an alternative approach, we used partially delipidated HDL ($_{pd}$HDL), in which the neutral lipids had been selectively extracted, rather than whole HDL as the starting material for reconstitution experiments. Importantly, such $_{pd}$HDL retained its specificity and potency; the time course of lysis by human $_{pd}$HDL was very similar to that of the initial whole HDL and bovine or mouse $_{pd}$HDL were non-lytic (**Fig. 3A**). Moreover, and unlike reported attempts with whole HDL (7), homologous reconstitution of apolipoproteins and polar lipids from human $_{pd}$HDL, and from mouse $_{pd}$HDL, produced particles with trypanolytic properties equivalent to the corresponding native $_{pd}$HDL (Fig. 3B). When heterologous recombinations of human apo$_{pd}$HDL with mouse or bovine $_{pd}$HDL-polar lipids were examined, their lytic effect did not differ from the homologous recombination (**Table 2**). By contrast, addition of mouse apo$_{pd}$HDL to human serum produced a marked reduction in trypanolytic activity, whilst bovine apo$_{pd}$HDL proved benign and human apo$_{pd}$HDL proved stimulatory (Table 2). Similar differential effects of these three apo$_{pd}$HDL preparations were also seen when the cytotoxic agent used was human HDL rather than serum (data not shown).

Susceptibility of transgenic mice to infection by *T. b. brucei*

A freshly prepared suspension of *T. b. brucei* (10^3 in 0.3 ml) was injected intraperitoneally into 20 transgenic mice (lines 19, 26, and 38), 16 control C57BL/6 mice, and 5 normal T/O mice and the animals were monitored daily

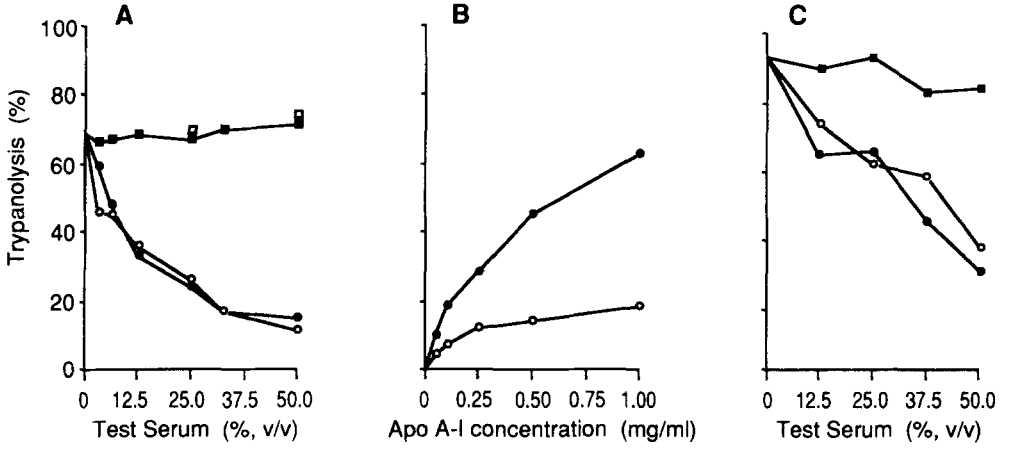

Fig. 2. Inhibition of human plasma or human apoA-I-mediated lysis in vitro of *T. b. brucei* by native or lipoprotein-depleted mouse serum. Trypanosomes (2.5×10^7/ml final concentration) were incubated at 37°C for 2 h with: (A) 25% (v/v) human plasma and increasing amounts of bovine plasma (■), sheep plasma (□), transgenic mouse serum (●), or control C57BL/6 serum (○); (B) increasing concentrations of human apoA-I in the presence (○) or absence (●) of 25% (v/v) normal T/O mouse serum; and (C) 25% (v/v) human plasma and increasing amounts of normal T/O mouse serum (●), apoB-free mouse serum (○) or lipoprotein-free mouse serum (■).

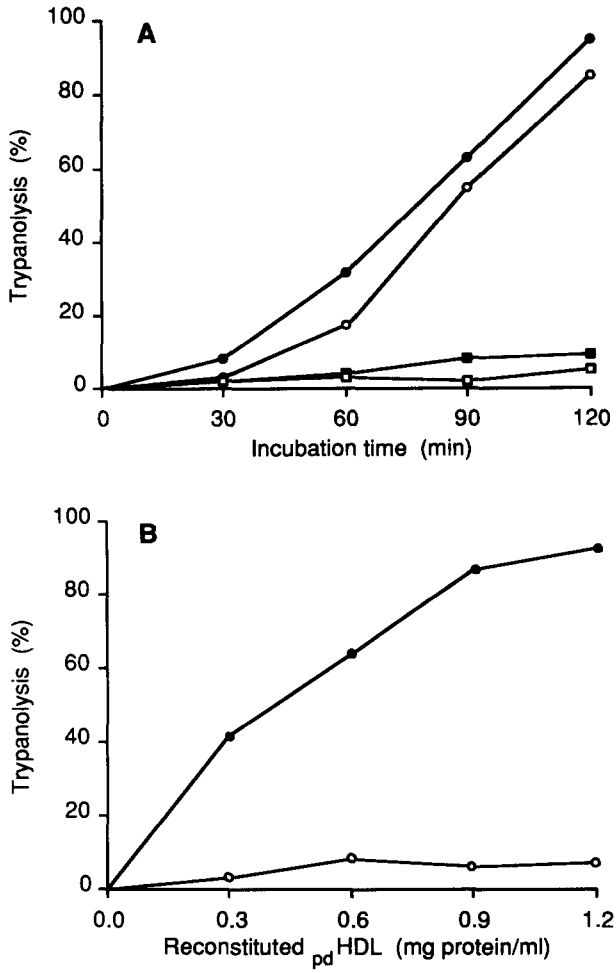

Fig. 3. Comparison of the lysis in vitro of *T. b. brucei* by partially delipidated HDL ($_{pd}$HDL) and by homologously reconstituted $_{pd}$HDL. Trypanosomes (2.5 × 10^7/ml final concentration) were incubated at 37°C (A) for increasing time with 1 mg protein/ml of whole human HDL (○) or of $_{pd}$HDL from human (●), mouse (■), and cattle (□) or (B) for 2 h with increasing concentrations of human apo$_{pd}$HDL (●) or mouse apo$_{pd}$HDL (○) reconstituted with homologous $_{pd}$HDL-polar lipids.

for the appearance of trypanosomes in their blood and for their survival. No difference either in the prepatent period of infection or in survival was apparent between the transgenic mice and the two groups of normal mice (**Fig. 4**).

DISCUSSION

Transgenic animals, most frequently mice, are increasingly being used to help answer a number of challenging questions in biology (32, 33). Potentially, the system also offers a unique opportunity in which to examine candidate trypanolytic proteins of human serum: expression of physiological levels of an active human protein in animal serum might be expected to impart cytotoxic properties to previously benign serum and, by analogy with the human situation, to confer species resistance to infection by the parasite. Therefore, in the present study, we have used transgenic mice in an attempt to obtain unequivocal evidence that a previously identified trypanolytic constituent of human HDL, its major protein apoA-I, was indeed an active factor.

In two independent experiments, carried out without prior knowledge of sample identity, trypanolysis in vitro by transgenic sera was greater than by control sera and was correlated significantly with the content of human apoA-I. We believe that these findings provide clear additional support for the trypanocidal capability of human apoA-I. By contrast, whilst the present work was already in progress, Rifkin (16) briefly reported that sera from another, independently produced, line of apoA-I transgenic mice lacked trypanolytic activity in vitro. However, apparently only two samples of transgenic mice sera were studied, suggesting that this discrepancy simply reflects the much greater number of animals used in our study rather than any inherent differences between the various lines.

Nevertheless, the trypanolytic activity in vitro of transgenic mouse serum was considerably weaker than that of human plasma, despite an equal or two- to threefold increase in the content of human apoA-I; indeed, trypanosomes pre-incubated with transgenic serum showed little loss of infectivity. One explanation for such weak activity is that apoA-I is not the sole trypanolytic factor of human HDL and, as proposed by others, that either a unique protein(s) present in a very minor HDL subclass is responsible (14) or that a particular combination of protein and phospholipids is essential (7). But such arguments tend, at best, to relegate apoA-I to a secondary role and do not adequately explain our previous experimental findings implicating apoA-I as the primary trypanolytic

TABLE 2. Effect of $_{pd}$HDL-polar lipids or apo$_{pd}$HDL from serum of normal mice on the trypanocidal action of human apo$_{pd}$HDL or human serum

Test Mixture[a]		% Trypanolysis
Human apo$_{pd}$HDL	+ human $_{pd}$HDL-polar lipids	84
	+ mouse $_{pd}$HDL-polar lipids	87
	+ bovine $_{pd}$HDL-polar lipids	85
Human serum	+ buffer	79
	+ human apo$_{pd}$HDL	98
	+ mouse apo$_{pd}$HDL	19
	+ bovine apo$_{pd}$HDL	82

[a]Incubations were carried out in duplicate and each tube of test mixture contained either human apo$_{pd}$HDL (1 mg/ml final concentration) complexed to the polar lipids isolated from the test $_{pd}$HDL (equivalent to 1 mg protein/ml) or human serum (25%, v/v) mixed with the test apo$_{pd}$HDL (1 mg/ml). Suspensions of trypanosomes (2.5 × 10^7/ml) were incubated with the various test mixtures for 2 h at 37°C. A second, independent experiment gave similar results.

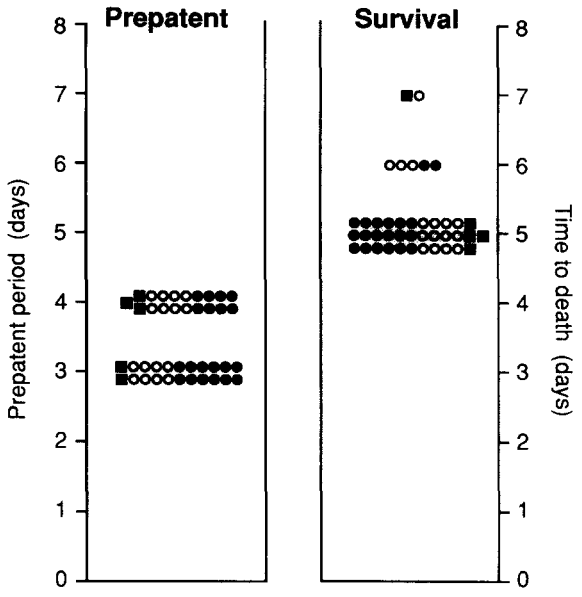

Fig. 4. Prepatent period and survival of transgenic mice inoculated with *T. b. brucei.* Normal T/O (■), control C57BL/6 (O), and transgenic (●) mice were injected intraperitoneally with 10^3 purified trypanosomes. The initial appearance of trypanosomes in the blood (prepatent period) and time of death were then recorded in days from the time of inoculation.

factor in human or baboon serum (15). Instead, we favor an alternative explanation: that human apoA-I is simply able to function less efficiently in mouse lipoproteins. Several possibilities are immediately suggested. ApoA-I is secreted into the circulation as a pro-apolipoprotein (pro-apoA-I) with a hexapeptide extension at its amino terminus; it is cleaved by a specific calcium-dependent protease in plasma to form mature apoA-I (34). Impaired conversion of human pro-apoA-I by the mouse protease would result in accumulation of pro-apoA-I in transgenic mouse serum and, conceivably, a lower cytotoxicity. In addition, expression of human apoA-I in mouse liver increases the size of serum HDL particles (35, 36) and also the amount of serum triglycerides (T. E. Hughes, M. E. Swanson, I. M. St. Denny, D. F. Nottage, D. S. France, J. R. Paterniti, Jr., and K. Burki, unpublished results) present, both of which may act to reduce overall lytic activity (8, 10, 15). Nevertheless, although there was evidence that all of these changes tended to occur in our lines of transgenic mice, particularly as the serum content of human apoA-I became very high (T. E. Hughes, unpublished observations), we considered that any accompanying loss of trypanolytic ability would be relatively minor. Accordingly, we sought to establish whether normal (and transgenic) mouse serum might contain a direct inhibitory agent.

Although excess mouse serum has been reported to inhibit the trypanocidal action in vitro of human serum (5), a much earlier study had found no effect (37). However, under our conditions, a clear and consistent inhibitory effect of mouse serum (including transgenic serum) on trypanolysis in vitro either by human serum or by purified human apoA-I was noted; further studies established that the antagonistic fraction in the serum was HDL. Recently, Rifkin (6) demonstrated that rat HDL, and to a lesser extent rabbit HDL, also have an inhibitory effect on the trypanolytic action of human serum. Moreover, by comparison of the phospholipid composition of lytic and non-lytic HDL and by addition of purified individual phospholipids to lytic baboon serum or HDL, she concluded that selected anionic phosphospholipids, particularly phosphatidylinositol but also phosphatidylserine and phosphatidylethanolamine, were important anti-trypanolytic agents. This concept is of clear interest; it offers a potential explanation for the weak cytotoxic action of transgenic mouse HDL. Nevertheless, our reconstitution experiments with $_{pd}$HDL established that phospholipid composition per se is unlikely to be important for inhibition of trypanolysis in vitro. Indirect evidence supports this conclusion: not only does inhibition by exogenous phosphatidylinositol require amounts at least 20-fold above the physiological level of about 10–15 μg/mg HDL protein (6) (and confirmed by our own studies; M. P. T. Gillett, and J. S. Owen, unpublished observations), but the original premise also appears flawed. Thus, whilst human and baboon HDL are considered lytic because their phosphatidylinositol/phosphatidylserine content is low (both 3.6% of total HDL-phospholipids compared to 5.2% and 7.8% in non-lytic rabbit and rat HDL) (6), the literature documents two non-lytic HDL fractions, sheep (15) and chimpanzee (38), that contain even lower levels, 0.4% and 0.9%, respectively (39). In contrast to this benign action of mouse apo$_{pd}$HDL-phospholipids, we found that the addition of mouse apo$_{pd}$HDL to human serum or to human HDL produced a marked reduction in trypanolysis in vitro. Thus, although depression of serum levels of mouse apoA-I, and probably of other HDL apolipoproteins (35), was a feature of our transgenic mice expressing high serum levels of human apoA-I, it appears that sufficient anti-trypanolytic endogenous mouse apolipoproteins remain with the HDL particles to limit their trypanocidal capability.

Although transgenic serum had only weak trypanolytic activity in vitro, it was possible that the mice might nevertheless prove resistant to infection by *T. b. brucei;* conceivably continuous exposure of the parasite to even a moderately inhospitable environment might be detrimental to multiplication or survival. Initially, an inoculum of 10^6 trypanosomes was used, but this produced a typical rising parasitemia and death in 2–3 days. Accordingly, a large series of animals was infected with only 10^3 cells but, as both the prepatent period of infection and survival were subsequently found to be indistinguishable between transgenic mice and control groups, it appeared that the

infections followed a normal time course and that the transgenic animals had full susceptibility. The slender, bloodstream forms of *T. b. brucei* used for the inoculation differ somewhat from the mature metacyclic trypanosomes deposited by the biting tsetse fly (40), but nevertheless it seems that even in vivo the balance between the actions of lytic human apoA-I and of anti-lytic mouse apolipoproteins in transgenic mouse serum is heavily weighted towards the latter. While not an aim of the present study, identification of the anti-trypanolytic apolipoprotein(s) in mouse HDL is of clear interest. First, because transgenic techniques also allow deletion of specific mRNA molecules (33, 41), suggesting that it should be possible to produce transgenic mice with benign serum; these could then be cross-bred with the human apoA-I transgenic mice. Second, because it may help explain an apparent differential effect of mouse serum towards human serum in vitro and in vivo. Thus, whilst mouse serum and rat serum (6), are effective inhibitors of human serum-mediated trypanolysis in vitro, even relatively small amounts of human serum (0.05–0.3 ml) rapidly clear trypanosomes when injected into heavily infected mice or rats (8, 10, 11). Intriguingly, it is almost 20 years since Hawking, Ramaden, and Whytock (5) and Hawking (42) suggested that two types of trypanocidal factor occur in human serum, one active in vitro and the other in vivo. Nevertheless, this observation has largely been ignored by other workers and its molecular basis has yet to be examined.

Finally, had the human apoA-I transgene conferred resistance to *T. b. brucei* infection on our mice, then a logical consequence, albeit one with serious economic and ecological considerations, would be to produce transgenic livestock animals for field testing in the tsetse fly regions of Africa. Unfortunately, because its sera contains endogenous factors inhibitory to human serum-mediated trypanolysis, it would now appear that the mouse is an inappropriate laboratory model for testing the human apoA-I transgene. However, such inhibitory factors were shown to be absent from bovine serum. This suggests that transgenic cattle expressing human apoA-I will be resistant to *T. b. brucei;* presumably, rearing of these cattle in endemic regions would not be restricted, potentially allowing the expansion of stocks in areas which are presently impoverished. Clearly, further progress towards such a long-term objective will require the selection of a suitable animal in which to critically test the human apoA-I transgene.

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