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Short communication

Distribution of apolipoprotein L-I and trypanosome lytic activity among primate sera

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Trypanosoma brucei brucei infects a wide range of mammals but is unable to infect humans because this *T. brucei* subspecies is lyzed by normal human serum (NHS). Other *T. brucei* subspecies (*T. brucei rhodesiense*, *T. brucei gambiense*) can resist this lytic activity and are therefore adapted to man, where they cause the disease known as sleeping sickness [1]. Studies of the mechanism conferring resistance of some trypanosomes to NHS have led to the identification of apolipoprotein L-I (apoL-I) as the lytic factor [2,3]. In a first step, resistance of *T. brucei rhodesiense* was shown to be due to a single protein termed serum-resistance associated (SRA) [2,4]. The N-terminal α-helix of this protein was then found to be necessary for its activity, probably because of its strong binding to the C-terminal α-helix of apoL-I, which takes place in the lysosome of the parasite [3]. Finally, apoL-I was directly shown to be able of trypanosome lytic activity [3].

ApoL-I was initially discovered as one of the lipoproteins bound to high-density lipoprotein (HDL) particles from human serum [5]. Subsequent work revealed that the *apoL-I* gene belongs to a multigene family, but is the only member encoding a N-terminal signal sequence necessary for secretion into the serum [6–8]. Limited evidence suggested the *apoL-I* gene arose in humans [8]. However, trypanosome lytic activity could also be observed in the serum of some nonhuman primates [9]. Interestingly, despite the close genetic and evolutionary relatedness of chimpanzees and man, chimpanzee sera were found to be devoid of trypanosome lytic activity [9].

In order to gain information about the evolutionary origin of apoL-I and its possible correlation with the appearance of trypanosome lytic activity in primates, we searched for the presence of apoL-I and trypanolytic activity in sera and plasma of various primate species, as listed in Table 1. Considering the proven existence of a multigene family consisting of very similar sequences [6,7], we did not approach this question by analysis of RNA sequences since the detection of *apoL-I*-like sequences would not be indicative of the presence of a functional serum lytic factor. Along the same line, detection by anti-apoL-I antibodies alone would not be sufficient neither because we could have expected the possible presence of related but nonfunctional proteins encoded by other *apoL* family members. Thus, our decisive criteria for the identification of apoL-I in primate sera were not only the detection of the typical Western blot pattern with the apoL-I specific antibodies, but also the binding of apoL-I to SRA.

A panel of 52 serum and plasma samples from 15 genera and 28 different species of primates was examined for the presence of apoL-I. As shown in Fig. 1 and summarized in Table 1, apart from man only a single primate species, *Gorilla gorilla*, possessed serum with the expected characteristics of apoL-I: a doublet of 42–39 kDa detected by anti-apoL-I antibodies and a specific binding to SRA-agarose. In all other primates, including chimpanzees, apoL-I was not detected. These characteristics were observed in all individuals tested in each species, and in the case of chimpanzees the negative results were obtained with two species, *Pan paniscus* and *Pan troglodytes*. Even prior to SRA chromatography no significant proteins were detected in these same primate samples with anti-apoL-I, the only bands observed being ascribable to known contaminants such as serumalbumin and endogenous antibodies [3] (data not shown).

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Table 1

Species[a]	Common name	Number of individuals	Lytic activity[b]	ApoL-I[c]
Homo sapiens	Man		+	+
P. paniscus	Pygmy chimpanzee (bonobo)	7	−	−
P. troglodytes	Central African chimpanzee	6	−	−
G. gorilla	Western lowland gorilla	5	+	+
Pongo pygmaeus	Bornean orangutan	4	−	−
Hylobates lar	White-handed gibbon	1	−	−
Hylobates leucogenys	White-cheeked gibbon	1	−	−
Hylobates syndactylus	Great gibbon (siamang)	2	−	−
P. papio	Guinea baboon	1	++	−
P. cynocephalus	Yellow baboon	1	++	−
P. anubis	Olive baboon	1	++	−
P. hamadryas	Sacred baboon	4	−	−
Theropithicus gelada	Gelada baboon	1	−	−
Lophocebus aterrimus	Black-crested mangabey	1	−	−
Mandrillus sphinx	Mandrill	3	−	−
Mandrillus leucophaeus	Drill	1	−	−
Cercocebus galeritus	Mangabey	1	−	−
Macaca nigra sulawesi	Macaque	1	−	−
Macaca silenus	Lion-tailed macaque	1	−	−
Macaca fuscata	Japanese macaque	1	−	−
Macaca sylvanus	Barbary macaque	1	−	−
Cercopithecus lhoesti	L'Hoest monkey	1	−	−
Cercopithecus mitis albolugaris	Syke's monkey	1	−	−
Cercopithecus neglectus	De Brazza's monkey	1	−	−
Cercopithecus diana	Diana monkey	1	−	−
Chlorocebus pygerythrus	Vervet monkey	1	−	−
Colobus angolensis palliatus	Colobus	1	−	−
Pygathrix nemaeus	Red-shanked Douc langur	1	−	−

[a] The species have been listed according to their relative evolutionary relatedness with man.

[b] Trypanosome lytic assays were performed on *T. brucei rhodesiense* clones ETat 1.2S and ETat 1.2R [2] as described in [3]. (+) Lysis of 1.2S, but not 1.2R; (++) lysis of both 1.2S and 1.2R.

[c] ApoL-I was revealed as described in the legend to Fig. 1.

The same nonhuman primate serum and plasma samples were also tested for trypanosome lytic activity. In order to detail the type of lysis, two different clones of *T. brucei rhodesiense* were used. These clones exhibit the same surface antigen (variant surface glycoprotein; VSG) termed ETat 1.2, but are either resistant (R) or sensitive (S) to NHS depending on the expression of SRA [2]. Thus, ETat 1.2S, but not ETat 1.2R, was expected to be lyzed by apoL-I [3]. Table 1 summarizes the results. Among the various primate sera tested only those from *G. gorilla* exhibited the expected lysis pattern. Thus, only sera from *Gorilla* and man shared both the presence of apoL-I and trypanosome lytic activity. As already mentioned in a previous publication [9], we found that some sera from *Papio* (baboon) also kill trypanosomes. Lytic sera were observed with *Papio papio*, *Papio cynocephalus*, *Papio anubis*, but not with *Papio hamadryas* (four samples tested) (Table 1). This lysis was clearly different from that induced by apoL-I, since it occurred with both ETat1.2R and 1.2S clones. In support to the idea that lysis by baboons is distinct from that due to apoL-I, a cross-reactive antigenicity has been noted between human and gorilla trypanocidal factors, whereas the baboon factor was found to be different [9]. In the same work [9], unusual incomplete lysis was seen with mandrill sera. We could not confirm this observation (Table 1). This discrepancy may be explained by the nature of the trypanosomes tested in this previous study [9] (a "monomorphic human serum-sensitive clone of *T. brucei gambiense*"). Resolution of these different lytic patterns will require additional investigation. Another study [8] detected *apoL-I*-specific sequences in the DNA from *Chlorocebus aethiops* (African green monkey), but this work ignored the existence of the *apoL-III* and *apoL-V* genes, which are more related to *apoL-I* in their 5′-extremities [6] than the *apoL-II* and *apoL-IV* genes targeted in the report [8]. Therefore, it is possible that the weak signal detected in African green monkey DNA by the 5′-specific probe from *apoL-I* originates from either *apoL-III* or *apoL-V*. It is unlikely that any of these sequences can produce a functional apoL protein, since they all lack the information necessary for the synthesis of the N-terminal signal peptide [6].

Our results confirm the unexpected observation that the serum from chimpanzee does not contain trypanolytic activity, despite its presence in gorilla serum and the closer genetic relatedness of man and chimpanzees compared to gorilla [10]. We show that apoL-I is absent from chimpanzee serum, which probably explains this observation. The reason for this absence is unknown and clearly worth further investigation. It can be hypothesized that the *apoL-I* gene arose in the common ancestor of gorillas and humans/chimpanzees [10] but following evolutionary divergence of humans and

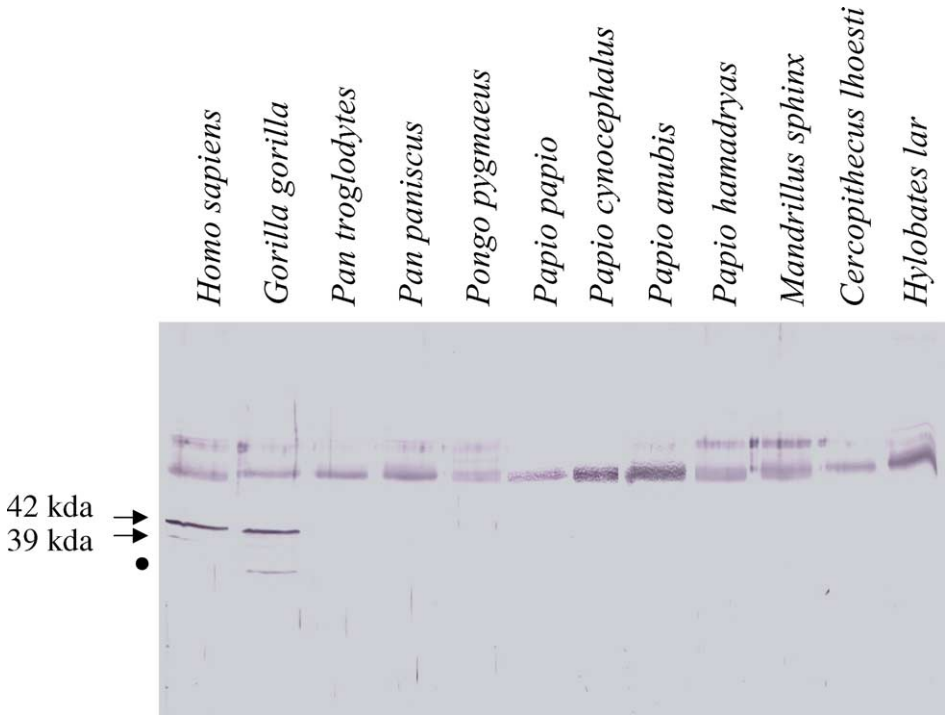

Fig. 1. Detection of apoL-I in the sera from various primates. Western blots of the fraction of the serum/plasma bound to SRA-agarose were incubated with polyclonal anti-apoL-I antibodies, exactly as described in [3]. The results obtained here are representative of those listed in Table 1. The arrows and dot, respectively, designate apoL-I and a frequent cleavage product of this protein. The components detected around 60 kDa include serum albumin and endogenous antibodies [3].

chimpanzees, this gene was either lost or was modified to become nonfunctional in the latter species.

Altogether these data further confirm that apoL-I is the trypanosome lytic factor of NHS [3]. One of the main arguments presented so far to support the previous candidate protein, haptoglobin-related protein or Hpr [11], namely its presence in gorillas but absence from chimpanzees because of a frameshift in the *Hpr* gene [12], is now challenged by the same observations for another serum protein, apoL-I.

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References

[1] Raper J, Portela MP, Lugli E, Frevert U, Tomlinson S. Trypanosome lytic factors: novel mediators of human innate immunity. Curr Opin Microbiol 2001;4:402–8.

[2] Xong HV, Vanhamme L, Chamekh M, et al. A *VSG* expression site-associated gene confers resistance to human serum in *Trypanosoma rhodesiense*. Cell 1998;95:839–46.

[3] Vanhamme L, Paturiaux-Hanocq F, Poelvoorde P, et al. Apolipoprotein L-I is the trypanosome lytic factor of human serum. Nature 2003;422:83–7.

[4] De Greef C, Imberechts H, Matthyssens G, Van Meirvenne N, Hamers R. A gene expressed only in serum-resistant variants of *Trypanosoma brucei rhodesiense*. Mol Biochem Parasitol 1989;36:169–76.

[5] Duchateau PN, Pullinger CR, Orellana RE, et al. Apolipoprotein L, a new human high density lipoprotein apolipoprotein expressed by the pancreas. Identification, cloning, characterization, and plasma distribution of apolipoprotein L. J Biol Chem 1997;272:25576–82.

[6] Page NM, Butlin DJ, Lomthaisong K, Lowry PJ. The human apolipoprotein L gene cluster: identification, classification, and sites of distribution. Genomics 2001;74:71–8.

[7] Duchateau PN, Pullinger CR, Cho MH, Eng C, Kane JP. Apolipoprotein L gene family: tissue-specific expression, splicing, promoter regions; discovery of a new gene. J Lipid Res 2001;42:620–30.

[8] Monajemi H, Fontijn RD, Pannekoek H, Horrevoets AJ. The apolipoprotein L gene cluster has emerged recently in evolution and is expressed in human vascular tissue. Genomics 2002;79:539–46.

[9] Seed JR, Sechelski JB, Loomis MR. A survey for a trypanocidal factor in primate sera. J Protozool 1990;37:393–400.

[10] Ruvolo M, Pan D, Zehr S, Goldberg T, Disotell TR, von Dornum M. Gene trees and hominoid phylogeny. Proc Natl Acad Sci USA 1994;91:8900–4.

[11] Smith AB, Esko JD, Hajduk SL. Killing of trypanosomes by the human haptoglobin-related protein. Science 1995;268:284–6.

[12] McEvoy SM, Maeda N. Complex events in the evolution of the haptoglobin gene cluster in primates. J Biol Chem 1988;263:15740–7.