Review Article

Prevention of fatal hepatic complication in schistosomiasis by inhibition of CETP

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Abstract

Schistosoma japonicum, once endemic all the East Asia, remains as a serious public health problem in certain regions. Ectopic egg embryonation in the liver causes granulomatosis and eventually fatal cirrhosis, so that prevention of this process is one of the keys to reduce its mortality. The embryonation requires cholesteryl ester from HDL of the host blood for egg yolk formation, and this reaction is impaired from the abnormal large HDL in genetic cholesteryl ester transfer protein (CETP) deficiency. When CETP was expressed in mice that otherwise lack this protein, granulomatosis of the liver was shown increased compared to the wild type upon infection of *Schistosoma japonicum*. The CETP deficiencies accumulated exclusively in East Asia, from Indochina to Siberia, so that Shistosomiasis can be a screening factor for this accumulation. CD36 related protein (CD36RP) was identified as a protein for this reaction, cloned from the cDNA library of *Schistosoma japonicum* with 1880-bp encoding 506 amino acids. The antibody against the extracellular loop of CD36RP inhibited cholesteryl ester uptake from HDL and suppressed egg embryonation in culture. Therefore, inhibition of CETP is a potential approach to prevent liver granulomatosis and thereby fatal liver cirrhosis in the infection of *Schistosoma japonicum*.

Keywords: Schistosoma japonicum, liver cirrhosis, granulomatosis, CETP deficiency, HDL, cholesteryl ester

Schistosoma japonicum in Asia

Schistosoma (S.) japonicum, an Asian specific blood fluke parasite, was endemic in East Asia including Indonesia, Philippines, China, and Japan as far as traced in recorded history. Its Mekong strain or *S. mekongi* is also found around the Mekong basin in Indochina peninsula. The parasite was first identified in Japan early 20th century when a few intensively infected regions were known, which is basically eliminated in these days[1]. It used to infect as many as 12 million people in China until the modern public health effort was initiated[2]. Although the number of the patients has dramatically decreased, it is still active at least in China[2–3], Philippines[4–5], Cambodia, Laos, Thailand, Malaysia, and Indonesia[6], perhaps accounting for some 2-million patients, and remains as the world second major Shcitosomiasis next to the African blood fluke, *S. mansoni*.

The life cycle of this parasite includes an intermediate host of specific fresh water snails such as *Oncomelenia nosophora*. The adults reside in the portal or intestinal veins of the host animals including humans, and lay eggs to be excreted with feces. The eggs embryonate

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in environmental fresh water and are hatched to release miracidia that penetrate into the snails to infect. They further grow there to sporcytes and then to cercariae, and are released into the water again to swim and penetrate the skin of the terminal hosts. Thus, endemic of *S. japonicum* is closely associated with intensive contact with natural fresh water reserve in everyday life. The historical geographic distribution of *S. japonicum* overlaps the regions with the culture of water farming of rice grain. This might have caused intensive endemic of *S. japonicum* in East Asia.

One of the most life threatening clinical manifestations is liver cirrhosis, which is caused by ectopic egg embryonation. The adult pairs of the parasite locate in the portal and its draining venules and lay eggs to be released to the intestinal tract. However, many of them are flushed back via the portal blood flow to the liver where they embolize and develop into miracidia, a phenomenon that leads to the morbidity and mortality of hepatic granulomatosis and cirrhosis[7]. Thus, egg embryonation is one of targets to prevent fatal development of schistosomiasis. The active mechanism to induce this lesion is unknown. Specific antibodies against various egg antigens have been identified as indicators of the infection, but their relationship with granulomatogenesis is unclear[8]. A potential pathogenesis factor is egg embryonation to the stage of miracidium, as the eggs only after this stage seem to cause the liver lesion when transplanted[9]. *L*-Selectin of the host binds the eggs only in the stage of miracidium[10]. Vaccination to stabilize the embryonation process has been proposed for an anti-Schistosomiasis therapy[11].

Schistosomas use the host plasma lipoproteins as lipid nutrient sources. *S. mansoni* express the low density lipoprotein (LDL) receptor-like protein[12–13]. The receptors for LDL and very low density lipoprotein (VLDL) were shown to mediate uptake of the lipoproteins in *S. mansoni*[14–15], as well as in *S. japonicum*[16–17]. However, there has not been much information whether and how high density lipoprotein (HDL) interacts with Schistosomas. We investigated a role of HDL in lifecycle of *S. japonicum*[18–20].

HDL for embryonation of *S. japonicum* eggs and CETP deficiency

Embryonation of *S japonicum* eggs to miracidia was investigated *in vitro*[19]. The paired adult parent parasites removed from the infected mouse portal vein were pre-incubated for laying eggs in the presence of 5% and 10% normal human serum for 2 days. The eggs were isolated and cultured for another 8 days for embryonaiton in the absence and presence of 10 % human serum. When the eggs were laid in the presence of adequate serum source (10%), they did not require serum for their embryonation. When they were laid in the poor nutritional condition (5% serum), the eggs needed the serum for embryonation (**Fig. 1A**). The finding indicates that the egg yolk is established for embryonation before they are laid but the eggs themselves also uptake nutrients from the serum, to grow. The latter effect was not reduced even when apoB lipoprotein was removed from the serum, indicating that LDL/VLDL are not major nutrient sources for the embryonation. Finally, HDL was shown as a requirement for embryonation (**Fig. 1B** and **C**). The results lead us to wonder how alteration of HDL metabolism influences the egg embryonation and accordingly development of the hepatic lesions.
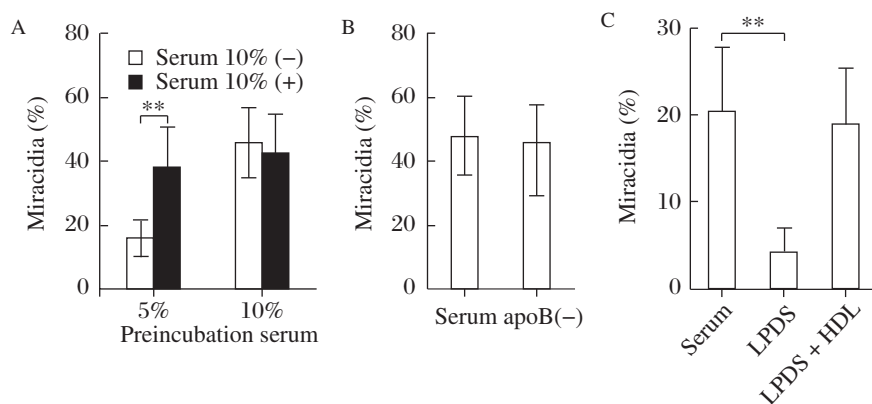
Fig. 1 Embryonation of *S. japonicum* eggs in culture, monitored as miracidia formation (%), modified from the reference[19] A: incubation of the eggs with and without human serum (10%) for 8 days after pre-exposure to 5 and 10 % human serum of the parents laying the eggs. B: Normal embryonation after the preincubation with 5 % serum, even with apoB-lipoprotein-deficient human serum (apoB (-)). C: Requirement of HDL for embyonaiton. LPDS; lipoprotein deficient serum. Asterisks ** indicate statistical difference at $P<0.01$ from serum (-) (A) or serum (C).
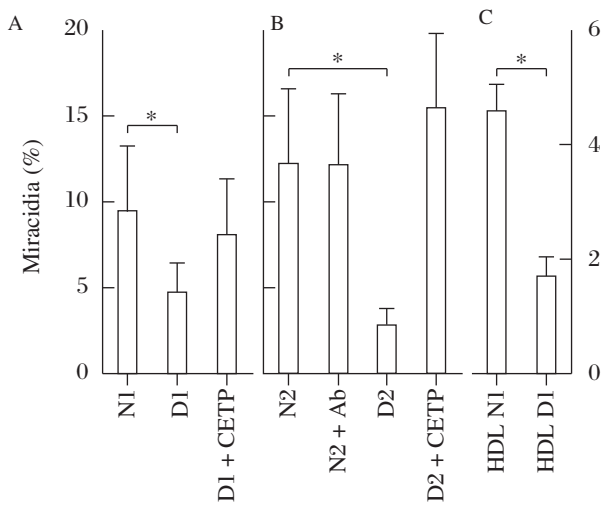
Fig. 2 Embryonation (% Miracidia) of *S. japonicum* eggs in culture with CETP-deficient human serum in 10% normal human sera (N1 and N2) and that of CETP-deficient subjects (D1 and D2), modified from the reference[18]. Embryonation is retarded in CETP-deficient serum (A) and adding CETP recovers this (B). Normal HDL is adequate for the embryonation but not HDL from CETP-deficiency (C). Asterisk indicates statistical difference at $P<0.05$ from normal HDL.

Some genetic mutations are known to manifest abnormal HDL metabolism. Deficiencies in HDL is seen in dysfunctional mutations in ATP-binding cassette transporter A1, lecithin: cholesterl acyltransferase and apolipoprotein AI, while deficiency of cholesteryl ester transfer protein (CETP) causes very high HDL cholesterol level as it generates a large abnormal HDL particle. While the former diseases are rare anywhere in the world except for the very limited regions with the founders' effect, CETP deficiency is found with very high prevalence widely in East Asia as discussed later in this text. Therefore, the in vitro experiments for egg embryonation were carried out with the serum from human subjects of genetic CETP deficiency[18].

The egg embryonation was significantly low when incubated with the serum of CETP deficient subjects, and this was substantially recovered in the presence of purified human CETP (***Fig. 2A***). However, the presence of the anti-CETP inhibitory antibody in the normal serum did not attenuate embryonation (***Fig. 2B***). Finally, normal HDL gave the eggs normal embryonation without other serum components, but HDL of CETP deficient subjects showed inadequate embryonation (***Fig. 2C***). Selective cholesteryl ester (CE) uptake was slower from HDL from CETP deficient subjects while free cholesterol exchange showed no difference between the normal and CETP-deficiency HDL (***Fig. 3***)[18]. Wild type mouse serum that lacks CETP activity did not induce adequate embryonation and
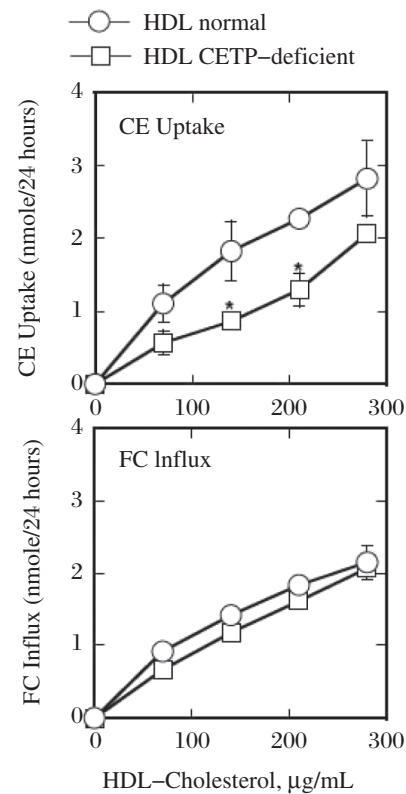

Fig. 3 Uptake of cholesteryl ester (CE) and free cholesterol (FC) from HDL by *S. japonicum* eggs, modified from reference[18]. The eggs were incubated with normal and CETP-deficient HDL with radiolabeled CE or FC.

the serum of the *CETP*-transgenic mouse enhanced it. Adding purified CETP to the mouse serum partially recovered the rate of embryonation (***Fig. 4***). Thus the findings with human blood serum were reproduced with mouse serum with and without CETP activity in vitro.

Accordingly, in-vivo studies were carried out in mice, using wildtype mice as a model for CETP-deficiency and the *CETP*-transgenic mice for normal human subject[18]. Number of the eggs plugged into the liver and the rate of their embryonation were counted microscopically in the liver specimens. While the number of the eggs was similar between the models, their embryonation was significantly higher in the transgenic mouse liver (***Fig. 5***). The average granulomatous lesion per egg was larger in the transgenic mice (***Table 1***). Absence of CETP thus seems to reduce ectopic embryonation of *S. japonicum* eggs in the host liver, making the host resistant to its hepatic complication.

Endemics of CETP deficiency

CETP is present in the plasma of certain species of mammalians including humans[21-24]. Human CETP is composed of 476 amino acid residues[25] with a glycosi-
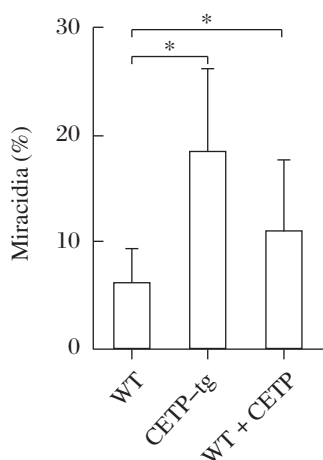
Fig. 4 The effect of mouse serum on egg embryonation (% miracidia), modified from the reference[18]. Embryonation is poor with wildtype mouse serum that lacks CETP and proceeds with *CETP*-transgenic mouse serum (CETP-tg). Adding human CETP to the wildtype serum partially restored embryonation. Asterisk indicates statistical difference at *P*<0.05 from WT.

***Table 1.* Granulomatous lesion in the liver.**

	CETP transgenic	Wildtype
Granuloma area (%)	10.5±5.5	14.4±5.4
Area per egg (μm²) × 10⁻³	86.7±26.5	66.1±19.3*

*P<0.05. Taken from the reference [18]. The eggs and granulomatous lesion were microscopically identified in the liver specimens of the wild-type and CETP transgenic mice, infected by *S. japonicum*. The total area of the lesion per egg was calculated for each section. The area per egg was calculated as an mean±SE of the 12 mice, for each of which 12 random liver sections were examined.

lated molecular weight of 74,000. It catalyzes non-directional equimolar exchange of CE and triglyceride (TG) among lipoproteins with low substrate specificity[26–29]. The reaction equalizes distribution of the core lipids among lipoproteins, and consequently causes the net move of CE from HDL to TG-rich lipoproteins such as VLDL and TG from TG-rich lipoproteins to HDL and LDL[26]. CE is generated in plasma on HDL and plasma TG is present in VLDL and chylomicron originating from the liver and the intestinal cells, so that the CETP reaction results in decrease of CE in HDL and increase of TG both in HDL and LDL. TG is hydrolyzed by hepatic lipase in any lipoprotein subfraction so that the size of HDL and LDL gets

smaller by the CETP reaction to produce HDL3 particles and so-to-speak small dense LDL. Increase in plasma TG indeed decreases plasma HDL and increases small dense LDL in the presence of CETP. In turn, HDL-CE increases when CETP reaction decreases[30].

Since generation of CE in HDL is one of the driving force for the removal of cell cholesterol[31], playing an important part of cholesterol transport for catabolism from peripheral somatic cells to the liver as its catabolic cite, the CETP reaction may facilitate this transport by sending HDL-CE to LDL for the pathway of recovery of LDL through the hepatic LDL receptor[32]. On the other hand, the increase in the HDL surface area in low CETP activity may provide more capacity to accept cell cholesterol.

CETP became a popular topic of lipoprotein study since discovery of the genetic defect of CETP. The patients were first described in Japan in 1985 as cases with hyperalphalipoproteinemia with the lack of CE transfer reaction between lipoproteins[33–34]. Its genetic background was soon established[35], and many cases were found thereafter in Japan. Two major mutations have been identified as major disorders, intron 14 G(+1)-to-A (Int14A) and exon 15 missense mutation
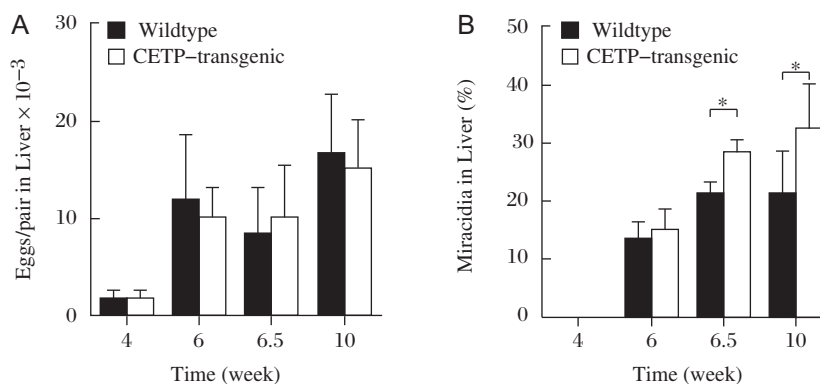
Fig. 5 Number of eggs embolizing in the liver and their ectopic embryonation in the mice infected with *S. japonica*, modified from the reference[18]. Wildtype and *CETP*-transgenic mice are infected by *S. japonica*. The ectopic embryonaiton is accelerated in the transgenic mice. A: Egg count in liver. B: Egg maturation in liver. Asterisk indicates statistical difference at *P*<0.05 from wildtype.

(D442G)[36–38]. Prevalence of these two mutants was found very high in Japanese general population, as int14A 1% to 2% and D442G 6% to 7%. In addition, sporadic cases with 10 or more other types of mutations were also identified among Japanese[39–41]. Accordingly, the estimated number of the heterozygotes can be around 10 millions in Japan and the homozygotes would be as many as 150,000 to 250,000. CETP deficiency may account for 27.6 % of the people with HDL cholesterol $\geqslant$ 60 mg/dL and 31.4%–32.5% of those with HDL $\geqslant$ 80 mg/dL in Japan[36,42]. Above all, Omagari of Akita district in Northern Japan was discovered as the region with high accumulation of the Int14A mutant, with the prevalence of the heterozygote of 27%[38]. Thus, genetic CETP deficiency was found highly common among Japanese.

The first non-Japanese patient was reported from Switzerland as a Chinese descent[43]. Several reports thereafter described CETP deficiency among other Asians.

Genetic prevalence of D442G mutant heterozygote case was found to be 3.3%–10.8% in the main-land Chinese population[44–46], and 4.5%–7.7% in the population of Taiwan[42,47–49]. It could be estimated up to 12% among Koreans based on its allele frequency of 6%[50]. Vietnamese D442G mutants were estimated to be 6.9% in the general population[51]. Nine cases were identified to be D442G heterozygotes out of the 35 individuals with hyperalphalipoproteinemias in Thailand[52], accounting for 26%, similar to the ratio among Japanese[36,42] strongly indicating CETP mutants common in Thailand. Further detailed information is available in the previous review article[53]. More recent result for elderly Siberian Yakuts indicates the prevalence of D442G mutant 16.2% in the native Yakuts and 5.2% among the non-indigenous[54], mostly Russians and Ukrainians, whose intermarriage rate with Yakuts may be 10 to 20% (Ariev AL, personal communication). Not much reliable information is available for the Int14A mutation, except for two out of the

Fig. 6 Endemics map of *S. japonicum* and CETP deficiency (D442G). Red spots show the regions where the cases were found since the parasite was identified in the early 20th Century. The regions spread over Japan, China including Taiwan, Mekong valleys, Thailand, Philippines, and Indonesia. The endemic was eliminated in many of these regions in the 21st Century. Data of the prevalence of G442G available in literature till today are listed as percentage in each ethnic general population unless otherwise indicated.
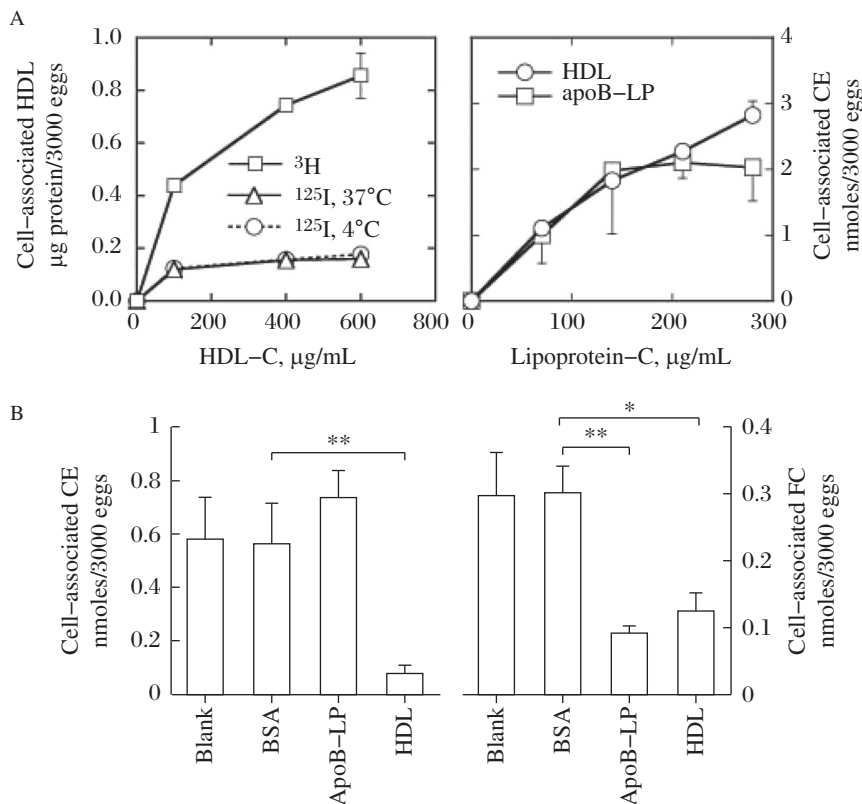
Fig. 7 Selective uptake of CE from HDL by the eggs of _S. japonicum_ modified from the reference[19]. A: The labeled HDL with ^{125}I for protein and with ^{3}H for CE was incubated with the eggs and the uptake of each radioactivity was measured and standardized for HDL protein (left). The CE uptake was also measured from HDL and LDL (right). B: CE (left) and FC (right) uptake from HDL in the presence of each plasma component indicated (BSA, ApoB lipoprotein, and HDL). Asterisks indicate statistical difference at $P<0.05$ (*) and $P<0.01$ (**) from BSA.

145 subjects (1.4%) in Hong Kong Chinese[18,47,55–59] and none of the 346 Vietnamese[51]. In a unique spot, Omagari of Akita district in Northern Japan, accumulation of Int14A mutant was found with the prevalence of the heterozygote 27.0%[38].

In contrast, CETP deficiency is rare in other ethnic groups. The first Caucasian case was reported in 1997[60], and one case of Int14A was reported in 1998 in Canada without ethnic background identification[61]. It was thus concluded that CETP deficiency is rare among North American Caucasians[62]. Nevertheless, a few studies reported sporadic cases of CETP deficiency in The United States[63], Italy[64–65], and the Netherlands[62,66]. There was an estimate of D442G mutant as "less than 1%"[67], but the ground for this estimation, as well as how "less", is unclear.

Clinical manifestation of CETP deficiency is limited to abnormal plasma lipoprotein profile represented by very high HDL cholesterol and moderately reduced LDL cholesterol. In the lack of CETP activity, CE generated in HDL is detained there, so that the core CE compartment expands and HDL particles become larger in their size, as large as LDL, and rich in apoE[68–71]. The patients in general do not exhibit any serious clinical symptoms. It has been wondered whether this large HDL is protective against atherosclerosis. The answer has been controversial for the genetic CETP deficiency[38,72–73] and the attempts of pharmacological inhibition of CETP were also inconclusive so far[74–75].

In summary, CETP deficiency is highly prevalent in East Asia, at least among Japanese, Chinese both in mainland and Taiwan, and Koreans, predominantly with the D442G mutant. It is likely similarly high in Thailand. The Int14A mutant may be the second common, but it is not clearly demonstrated except for Japan. Other types of mutation have also been found frequently in Japan. It may also be so in other East Asian regions, but the information is not adequate. In contrast, this disease is rare in other ethnic groups.

For geographic or ethnic accumulation of a genetic abnormality, two potential hypotheses may be considered, "founders' effect" or screening by a regional fatal disease(s). The former cases are normally found in limited communities of the descendants of earlier settlers. Typical examples are accumulation of familial hypercholesterolemia in French Canadians[76],

Afrikaners in South Africa[77] and perhaps in Lebanon[78]. In this case, the accumulated mutations may not be highly diverse as originating in a few carrier families. The latter is represented by sickle cell anemia that is resistant to malaria infection, as a believed reason for high prevalence of this genetic anemia among African ethnic groups[79–81]. This case may affect large populations historically exposed to such diseases, mostly infectious diseases.

Accumulation of CETP deficiency can also be discussed from such points of view. Majority of the patients of the region may be limited to one or two type(s) of mutations although further diversity of the mutation is also observed in the region, being not inconsistent with founders' effect. However, the region where this disease is found with high prevalence seems a large portion of East Asia, far beyond founder's effect. No specific settler family can be conceivable to account for such large descending population affected. However, extreme accumulation of int14A in Omagari may be the case of local "founders' effect"[38]. It should be noted that its only significant clinical phenotype is abnormal plasma lipoprotein metabolism. Very few infectious diseases are found with any relation to or dependency on plasma lipoproteins. Schistosomas may be one of the few to meet such criteria. The regions of historic endemics of *S. japonicum* and of high prevalence of CETP deficiency in fact largely overlap (***Fig. 6***).

Identification and characterization of CD36-related protein in *S. japonicum*

In order to investigate a specific role of HDL for egg embryonation, uptake by the *S. japonicum* eggs of cholesterol, the most specific nutrient carried by lipoproteins, was observed. Uptake of lipoprotein CE by the eggs is shown in ***Fig. 7***[19]. CE is selectively taken up from HDL. The uptake also seems to occur from apoB-lipoprotein, but it is saturated at much lower level and the pathways are independent of each other. In contrast, free cholesterol (FC) exchange between the eggs and lipoproteins is by a common pathway for HDL and apoB-lipoproteins (***Fig. 7***).

Selective cellular uptake of CE has been shown to be mediated by CD36-like proteins, such as scavenger receptor-B1 (SR-B1) in rodent[82] or CLA1 in human[83]. Assuming a similar protein mediates the reaction, expression of mRNA was searched in *S. japonicum* by using the 489-bp probe derived from the cDNA of Sj-Ts2 protein that has one of the CD36 domains (671-bp submitted and registered as Genbank AF291715). From the *S. japonicum* adult cDNA library, Sj-Ts2-containing cDNAs of variable sizes longer than 1-kb were obtained all seemingly derived by single transcription, including the sequence of the reported Sj-Ts2 protein. The sequence of the full length (1880-bp) original mRNA was determined and deduced to 506 amino acid residues (***Fig. 8***) (GenBank accession no. AY496973)[19]. Though there is an indication of alternative splicing of this mRNA, the size of the PCR product, with the first-strand cDNA derived from total RNA as template, was similar (467-bp) between eggs and adults (***Fig. 9***). Therefore, the message is likely to be expressed in eggs though the level seems lower.

The amino acid sequence indicated that it has two transmembrane regions and at least three CD36 domains that are conserved in 32 CD36 proteins from variety of organism according to Conserved Domain search analysis (***Fig. 8***), having high homology with rat SR-B1, rat CD36 and human CLA-1. The protein was thus thought to be of the CD36 family and termed CD36-related protein (CD36RP)[19]. It had 15 nucleotide polymorphism sites in the coding region identified during screening, resulting in 11 amino acid substitutions. Three *N*-glycosylation sites (aa97, 205, 248) identified among the 15 candidates asparagine in CD36RP were conserved in mammalian SR-BI and CD36 (***Fig. 8***). In fact, the size of CD36RP in the *S. japonicum* adult decreased from 82 kDa to 62 kDa after *N*-glycanase treatment[19].

A recombinant peptide representing the extracellular domain containing the conserved Cys and Pro-rich domain of CD36RP was used for binding to lipoproteins[19]. The water-soluble extracellular domain peptide was shown to strongly bind to normal HDL, but neither to LDL nor to the HDL from a CETP deficient subject as poorly as to LDL (***Fig. 10***)[19]. The antibody against the extracellular domain peptide of CD36RP significantly suppressed the active CE uptake at 37°C, as well as the egg embryonation to miracidia (***Fig. 11***)[19].

Proposal of the hypothesis

Available information can be summarized as follows. 1) CETP deficiency is a unique inborn error to manifest very high HDL cholesterol due to extreme enlargement of HDL particles, without other apparent clinical features. CETP deficiency was shown highly prevalent among East Asians, at least shown with those in Japan, mainland China, Taiwan, Vietnam, Korea, Siberia and probably Thailand, while it seems rare among other ethnic groups. 2) One of the regional endemic diseases having any relation to plasma lipoproteins is schistosomiasis where the parasites use plasma lipoproteins as nutrient sources. *S japonicum* has been known evidently endemic at least in Japan, China, Philippine, Indonesia, and its close

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   1  aca ttt tat tta aca gct ttt aaa aat taa cag aca acg ttt cac ttg tac acg ATG ATA   60
                                                                                  M   I
  61  TCT CGT GTT TGG TTA AGT GCT ACA GTT TTA TTT ACT TTG TTG ATT TGT ATA TCA TTA TTA  120
       S   R   V   W   L   S   A   T   V   L   F   T   V   L   I   C   I   S   L   L
 121  TCT TTA TGC GTA TTA CAA CCG TTT CTT TGG TTT CTA ATC AAT AGG CAA ACA CGT TTA ACT  180
       S   L   C   V   L   Q   P   F   L   W   F   L   I   N   R   Q   T   R   L   T
 181  CCA GGA ACT AAA CTG TAT TCA GAA TGG TTA GAG CCA TCA CTA CCT GTT TTA ACA CAA TTC  240
       P   G   T   K   L   Y   S   E   W   L   E   P   S  (L)  P   V   L   T   Q   F
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 301  CAA TTA GGA CCA TAT ACA TAC CGT GAA AAA CGT TTG AAG CTT AAT ATT ACA CAT AGT AAT  360
       Q   L   G   P   Y   T   Y   R   E   K   R   L   K   L   N   I   T   H   S   N
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       G   T   I   T   Y   K   E   M   K   W   Y   Y   F   D   Q   N   L   S   N   G
 421  ATG GTG AAT GAT TCA ATC ACT AGT GTC AAT CTT GTT TTC ATA TCG ATT GCA TTG AGA ATC  480
       M   V   N   D   S   I   T   S   V   N   L   V   F   I   S   I   A   L   R   I
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       N   S   M   P   W   F   L   K   Q   I   I   E   L   I   E   S   R   F   H   E
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       Y   L   F   I   T   K   T   V   N   E   L   L   W   G   Y   N   D   E   L   L
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       T   Y  (L)  S  (T)  H   G   F   N   M   S   T   V   T   H   I   G   L   F   I
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       N   K   N   N   T   L   S   D   Y   V   T   I   N   D   G   L   H   N   N   K
 721  ATG ATT GGA CAA ATT ACT CGA TAT CAT GGT AAT ACA ACA TTA TCC TAT TGG AAT AGT TCA  780
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       T   A   N   M   I   N   G   S   D   G   T   F   F   H   S   F   L   T   K   Y
                                      IPB002159D
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       D   G   V   L   D   M   S   S   C   Q   P   G   A   P   I   V   V   S   Q   P
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       S   T   T  (L)  L   P   I   V   F   I   N   E   S   V   Q   L   N   D   T  (L)
1321  ATT GAA CAA TTG ACT AAT GCA TTA ATT CAA CAA CCA TTC ATT GTT CAA ACA ATT TTA GTT 1380
       I   E   Q   L   T   N   A   L   I   Q   Q   P   F   I   V   Q   T   I   L   V
1381  TGT ATT ATC ATT TTG TCA ATA ATT TCA CTA GGT TCA CTT ATT TCA ATA CAT TTT TAT CAG 1440
       C   I   I   T   L   S   I   I   S   L   G   S   L   I   S   I   H   F   Y   Q
1441  AAT AGA CAA CAT ACT ACT TAT ATG CAT TTT ATT GAT AGC CAT CAA TCC AAT GAT GTT ATA 1500
       N   R   Q   H   T   T   Y   M  (H) (H)  F   I   D   S   H   Q   S   N   D   V   I
1501  CCT CAG AAT ACA TTA GAG GTT AAT ACA CAA CAA CAG ACA ATA TCA AAT GAT TTA CAA GAA 1560
       P   Q   N  (S)  L   E   V   N   T   Q   Q   Q   T   I   S   N   D   L   Q   E
1561  AAT CCA ATT GTT TAA ttg tta att aac atg att caa ttg aca ata ttg tca tta tat gtt 1620
       N   P   I   V   *
1621  tgt tat tgt tat tgt tct ata ttt gca tct gac tat cga tta gac att gat tag tga aag 1680
1681  tct gtg tgt gag tgt gtg taa ttt tca att gtc aat ttt ttg taa ctt aga tgt ttt act 1740
1741  tgt gaa tta ttt aat tta cct tca agg aga ata atc act gat tat ttg atg aat tgt gca 1800
1801  taa caa ttg aat aaa tga caa aat atc tac agt taa gat gaa tga aat acc att gag ttt 1860
1861  att gaa ctc tct atg agg taa aaa aaa aaa  aa                                      1892
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***Fig. 8* Nucleotide sequence and the deduced amino acid residue sequence of CD36RP cloned from *S. japonicum*, modified from the reference[19].** Shadowed portions are predicted membrane spanning regions. Bald letters of N indicate potential glycosylation sites. The sequences double underline portions are potential CD36-related regions (IPB 002159D, 002159E, and 002159F).
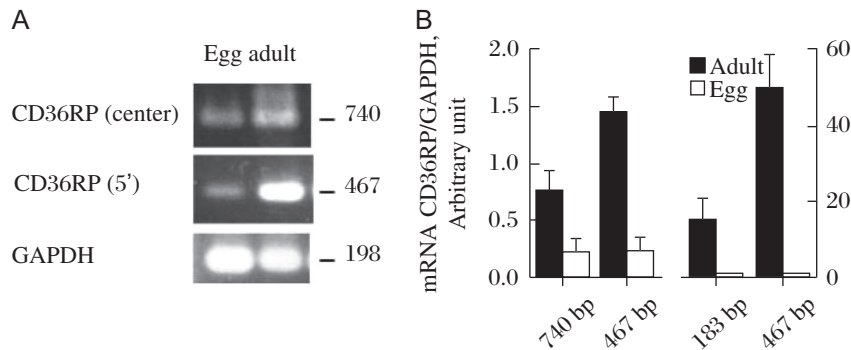
Fig. 9 Expression of CD36RP in *S. japonicum*, the adults and the eggs, modified from the reference[19]. Two different probes (near the center and 5′ region) were used to yield PCR products of 740 bp and 467 bp, respectively. A: The results of conventional PCR. B: The results of real-time PCR.

strain in the Mekong basin, substantially overlapping with the regions of high prevalence of the CETP deficiency (***Fig. 6***). 3) The ectopic egg embryonation to miracidia of *S japonicum* in the liver requires normal plasma HDL, and it is impaired with the HDL of CETP deficiency. 4) CD36RP, a membrane protein of the CD36 family, was identified in *S japonicum*. The antibodies against the extracellular domain of this protein suppressed selective uptake of CE by the eggs as well as the embryonation of the eggs.

Based on these findings and insights into them, we propose that CD36RP is a candidate for a mediator of selective uptake of CE from HDL by *S. japonicum* necessary for the egg embryonation to miracidia[19–20]. Pre-exposure of the adult schistosomes to wildtype HDL is sufficient for the eggs to mature, perhaps because the yolk of the egg was preformed adequately in such a condition. The eggs laid with inadequate yolk may still mature provided that normal HDL is supplied after they are laid. Absence of normal HDL retards embryonation of the *S. japonicum* eggs in the host liver and, accordingly, prevents hepatic granulomatosis, in a situation such as CETP deficiency where abnormal large HDL does not efficiently bind CD36RP. Therefore, it is not unreasonable to speculate that CD36RP is a strong candidate for a mediator of HDL-CE uptake by the adults and eggs of *S. japonicum* as a key molecule for embryonation of the egg to the miracidium, based on structural similarity of CD36RP to CD36 or SR-BI, selective binding of the extracellular domain of CD36RP to HDL, and suppression of the HDL-CE uptake and embryonation of the eggs in culture by the antibody against CD36RP. CD36RP and host plasma HDL are both key elements for hepatic granulomatosis in *S. japonicum* infection, a fatal pathological process in infected pateints.

If this hypothesis is valid, inhibition of CETP could be useful to prevent hepatic granulomatosis in schistosomiasis. CETP inhibitors have been developed hoping to raise HDL cholesterol to prevent athero-
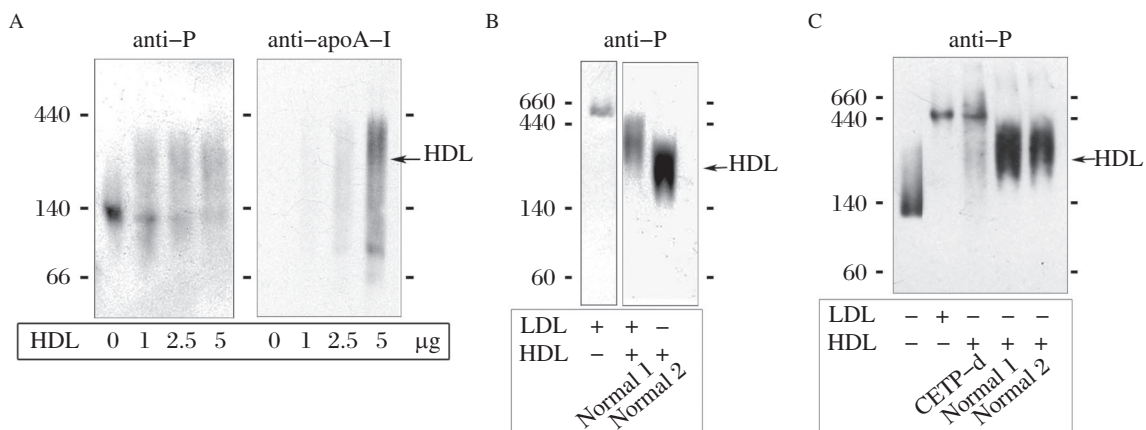
Fig. 10 Interaction of the peptide representing the extracellular domain of CD36RP (residues 249–369) with lipoproteins, modified from the reference[19]. HDL was visualized by immunoblotting with anti-apoA-I antibody and the peptide was visualized by the antibody against the shorter peptide (331–348)(anti-P). A: Binding of the peptide with human HDL. B: Interaction of the peptide with HDL and LDL. C: Binding of the peptide with HDL from the normal and the CETP-deficient subjects.
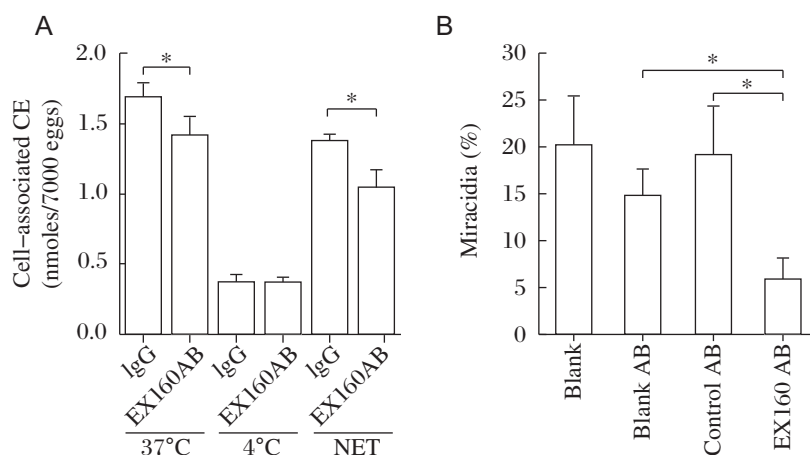
Fig. 11 Suppression of CE uptake by and embryonation of the *S. japonicum* eggs in culture, by the antibody raised against the extracellular domain peptide of CD36RP representing the residues 249–408, modified from the reference[19]. Blank AB, nonimmunized rabbit antibody, Control AB, antibody against the intracellular domain of CD36RP (residues 331–348). Asterisk indicates statistical difference at $P<0.05$ from IgG or Blank AB/Control AB.

sclerotic vascular diseases such as coronary heart disease. The hypothesis has been proven valid in an animal experiment[84], but has not been successful to demonstrate clinical outcome yet[74–75]. The inhibitors, however, can be useful to intend the off-target effect on other serious public health problem, prevention of the fatal liver cirrhosis in Schitosomiasis[20].

Acknowledgements

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