Rheological and functional impairments in cholesterol-loaded human erythrocytes

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Summary. Decreased deformability, slightly increased suspension viscosity and retarded oxygen egress were observed in cholesterol-loaded, human erythrocytes. These functional changes resulted from the decreased membrane fluidity induced by cholesterol.

It is well known that cholesterol molecules in the phospholipid bilayer modify the membrane phase, and decrease membrane fluidity[1-3]. In the human erythrocyte membrane, Cooper et al. have demonstrated increased resistance against osmotic hemolysis[4] and decreased 'membrane viscosity'[5] as the membrane cholesterol increased. Here, we report other functional changes induced by cholesterol-loading.

The cholesterol-loaded human erythrocytes were prepared by the method of Cooper et al.[5], then washed with the isotonic phosphate buffered saline solution 3 times. The membrane lipids were quantified by $CHCl_3$-methanol extraction[6]; the amount of cholesterol was measured by gas-chromatography[7] and that of phospholipids by phosphate assay[8].

The 'deformability' was measured by a micropipette aspiration technique, in a manner essentially similar to Braasch's method[9]: the sedimented, individual cells on a slide glass were aspirated through a micropipette (of which the inner diameter of the orifice $\simeq 3$ µm) by applying negative pressure (circa 10 cm H_2O), and the rates of decrease of electrical current due to the cell aspiration, recorded on a pen-oscillograph, were taken as the parameters of the individual 'deformability' (which expressed the 'easiness' of entering into the small orifice). The viscosity of the erythrocyte suspension was determined by a cone-plate viscometer

Table 1. Effect of the membrane cholesterol on the viscosity and deformability of erythrocyte suspensions

Sample No.		1	2	3
Cholesterol (C)	[$\times 10^{-10}$ µmoles/cell]	2.70	3.01	6.96
Phospholipids (P)	[$\times 10^{-10}$ µmoles/cell]	4.06	3.38	3.86
C/P	[molar ratio]	0.67	0.89	1.80
MCV	[µm^3]	96.8	92.1	96.5
MCH	[$\times 10^{-12}$ g/cell]	32.9	30.5	32.2
ATP[a]	[$\times 10^{-3}$ M/l packed cells]	1.65	1.65	1.56
ADP[a]	[$\times 10^{-4}$ M/l packed cells]	1.28	1.21	1.23
AMP[a]	[$\times 10^{-5}$ M/l packed cells]	1.44	1.63	1.59
2,3-DPG[b]	[$\times 10^{-3}$ M/l packed cells]	4.89	5.02	5.04
P_{50} (n)[c]	[mm Hg]	29.5 (2.77)	30.4 (2.78)	29.8 (2.76)
pH_{in}[d]		7.06	7.07	7.06
Deformability[e]	[relative value]	1.07 ± 0.14	1.00 ± 0.21	0.85 ± 0.11
η_{app}[f] [cP]	(at $\gamma = 376.1$ sec^{-1})	4.17	4.17	4.53
	(at $\gamma = 150.5$ sec^{-1})	4.84	4.84	5.29
	(at $\gamma = 75.2$ sec^{-1})	5.62	5.68	6.10
	(at $\gamma = 37.6$ sec^{-1})	6.40	6.52	7.11
	(at $\gamma = 18.8$ sec^{-1})	7.53	7.65	8.13
	(at $\gamma = 3.76$ sec^{-1})	11.95	11.95	11.95

[a] Determined for the acid extracts of erythrocytes by liquid chromatography. [b] 2,3-diphosphoglycerate, determined by the method of Maeda et al.[12]. [c] The oxygen tension at 50% oxygenation (P_{50}) and the Hill's coefficient (n, in the parenthesis), measured at 37 °C in the phosphate buffered saline (pH 7.38, CO_2 depleted). [d] Intracellular pH, measured by the method of Enoki et al.[13]. [e] See text. Averaged values, measured at 34 °C. [f] Apparent viscosity of the erythrocyte suspension (Ht = 55%) in the phosphate buffered saline (pH 7.40, at 37 °C).

Table 2. Effect of the membrane cholesterol on the velocity of oxygen egress and the deformability

Sample No.		1	2	3	4
Cholesterol (C)	[$\times 10^{-10}$ µmoles/cell]	3.78	4.42	5.90	7.20
Phospholipids (P)	[$\times 10^{-10}$ µmoles/cell]	3.93	3.84	4.06	4.17
C/P	[molar ratio]	0.96	1.15	1.45	1.73
Haemoglobin(Hb)	[$\times 10^{-3}$ M/l packed cells]	5.80	5.85	5.25	5.76
2,3-DPG	[$\times 10^{-3}$ M/l packed cells]	3.07	2.97	2.63	3.21
2,3-DPG/Hb	[molar ratio]	0.53	0.51	0.50	0.56
P_{50} (n)*	[mm Hg]	23.0 (2.62)	24.6 (2.65)	22.8 (2.65)	23.8 (2.65)
pH_{in}*		7.05	7.03	7.05	7.11
k_c**	[sec^{-1}]	3.31 ± 0.08	3.25 ± 0.07	3.02 ± 0.10	3.03 ± 0.07
Deformability*	[relative value]	1.00 ± 0.16	0.97 ± 0.18	0.91 ± 0.12	0.79 ± 0.15

* See table 1. ** The velocity constant of haemoglobin deoxygenation[10,11], measured in the phosphate buffered saline, pH 7.42, at 20.5 °C.

with a 0.8° cone attached (Tokyo Keiki Co.). The kinetic parameter of hemoglobin deoxygenation (the velocity constant, k_c) upon mixing the air-saturated erythrocytes with the hydrosulfite solution[10,11] was measured by a stopped flow apparatus (Union Giken Co.).

The representative results are summarized in tables 1 and 2. The 'deformability' decreases as the cholesterol/phospholipid ratio increases. A slight but definite increase of the viscosity of the cell suspension, in particular at higher shear rates, is noticed with the cholesterol-loaded erythrocytes (table 1). In a separate experiment, decreased motion of a stearate spin label has been observed in the cholesterol-loaded erythrocyte membrane[14].

Furthermore, the velocity constant of oxyhemoglobin dissociation was reduced, as the membrane cholesterol was augmented (table 2), in spite of the similarity a) of the oxygen equilibrium curve (as expressed by P_{50} and n), b) of the cell shape (e.g., MCV and MCH, also the microscopic observation) and c) of the amounts of the allosteric effectors (such as 2,3-diphosphoglycerate, ATP and intracellular pH; though the amounts of 2,3-diphosphoglycerate decreased during the sample preparation in this case).

Increase in membrane cholesterol 1. immobilizes the acyl chain motion of the phospholipids and reduces the membrane fluidity[1-3], and 2. may change the membrane protein organization[15], therefore the erythrocyte deformability decreases. The slight increase of the suspension viscosity under higher shear rates, observed with the cholesterol-loaded cells, may be explained by the decreased ability of passive deformation to reduce the rheological resistance. The increased blood viscosity observed with hyperlipoproteinemias[16], might be due to the increased membrane cholesterol.

The retarded rate of oxygen egress from the cholesterol-loaded erythrocytes may result from the decreased rate of oxygen diffusion in the lipid portion of the membrane, since Fischkoff and Vanderkooi[17] have shown that the diffusion rate of oxygen molecules is decreased in the cholesterol-containing phospholipid vesicles.

In conclusion, an increase in erythrocyte membrane cholesterol not only affects the rheological properties such as deformability and suspension viscosity, but also reduces the rate of oxygen egress from the cells.

1 D. Chapman, Q. Rev. Biophys. *8*, 185 (1975).
2 R.A. Demel and B. de Kruyff, Biochim. biophys. Acta, *457*, 109 (1976).
3 T.J. McIntosh, Biochim. biophys. Acta, *513*, 43 (1978).
4 R.A. Cooper and J.H. Jandl, J. clin. Invest. *47*, 809 (1968).
5 R.A. Cooper, M.H. Leslie, S. Fischkoff, M. Shinitzky and S. Shattil, Biochemistry *17*, 327 (1978).
6 J. Folch, M. Lee and G.H.M. Stanley, J. biol. Chem. *226*, 497 (1957).
7 N. Ikegami, M. Mitsui and K. Sato, Jap. J. exp. Med. *41*, 163 (1971).
8 G.R. Bartlett, J. biol. Chem. *234*, 466 (1959).
9 D. Braasch, Pflügers Arch. *329*, 167 (1971).
10 F.J.W. Roughton, Br. med. Bull. *19*, 80 (1963).
11 J.A. Sirs, Biochim. biophys. Acta *112*, 538 (1966).
12 N. Maeda, H. Chang, R. Benesch and R.E. Benesch, New Engl. J. Med. *284*, 1239 (1971).
13 Y. Enoki, S. Tomita, N. Maeda, M. Kawase and T. Okuda, J. physiol. Soc. Japan *34*, 761 (1972).
14 T. Suda, N. Maeda, M. Sekiya, K. Matsuoka, R. Tokita and T. Shiga, Med. J. Osaka Univ. *29*, 21 (1978).
15 H. Borochov, R.E. Abott, D. Schachter and M. Shinitzky, Biochemistry *18*, 251 (1979).
16 H. Leonhardt and H.-R. Arntz, Klin. Wsch. *56*, 271 (1978).
17 S. Fischkoff and J.M. Vanderkooi, J. gen. Physiol. *65*, 663 (1975).

Hymenolepis nana: Transfer of acquired immunity in mice through sensitized peritoneal exudate cells

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Summary. Sensitized peritoneal exudate cells from syngeneic donor Swiss albino mice infected with single and repeated doses of viable eggs of *Hymenolepis nana* produced a significant adoptive immunity in test mice when compared with animals which received non-sensitized (normal) cells. No significant difference was observed among the 2 recipient groups which received singly or repeatedly sensitized peritoneal exudate cells.

Hymenolepis nana, (the dwarf tapeworm) is known to be highly immunogenic[2], and in the mouse immunity can be detected at various intervals after primary infection[3]. Reports of passive transfer of immunity directed against *H. nana* from infected donors to uninfected animals are very few. Male C_3 B F_1/cum mice irradiated after normal expulsion of adult *H. nana* worms and injected with syngeneic normal bone marrow cells showed persistance of acquired immunity 1 day after irradiation[4]. The role of CMI was fully established when Friedberg et al.[5] demonstrated transfer of acquired immunity to X-irradiated recipients through immunized spleen cells from previously infected donors.

While the present authors were engaged in investigations involving CMI in the *H. nana* mouse model, it was found that sensitized peritoneal exudate cells from infected donors produced a measurable amount of acquired immunity in syngeneic recipients challenged with *H. nana* eggs.

Materials and methods. Collection and transfer of peritoneal exudate cells were in accordance with the method of Vardhani and Johri[6]. Approximately 26×10^4 cells were transferrred within 4 h of collection. Syngeneic female recipient Swiss albino mice, of approximately the same body weight and age (6-8 weeks and 15-20 g) were divided into 5 groups of 30 mice each. Groups a and b received cells from singly, d and c from doubly infected and e from uninfected (control) donors. These recipient groups were further divided into 3 subgroups, each challenged with a single dose of 1000 *H. nana* eggs at 7, 14 and 21 days after cell transfer. Infection of donor mice, injection of sensitized cells, and challenge were carried out according to table 1. Mice were necropsied on the 4th day (96 h) after challenge.