The Iraqi J. Vet. Med. 14 (1990)

A HISTOCHEMICAL STUDY ON THE CIRCULATING LIPOPHAGES IN CHOLESTEROL FED GUINEA PIGS

T.H. Al-Shebeb, H.S. Al-Nassir[2], N.A. Jaber[3]

University of Baghdad, College of Veterinary Medicine, Department of Pathology[1], Department of Veterinary Public Health[2] and Department of Veterinary Medicine[3], College of Veterinary Medicine, University of Baghdad.

SUMMARY

The postulation that circulating lipophages in cholesterol-fed guinea pigs may originate from lipidladen splenic macrophages was tested. The experimental animals were allocated in two groups: high-cholesterol (HC) and control (C) groups. Wright-Giemsa, Oil-red O, non specific estrase and Perl's prussian blue stains were performed on peripheral blood films on both day-30 and 60 of the experiment. Vacuolated leukocytes were demonstrated by day 60 in the HC group and proven to be lipid-laden monocytes. No hemosiderin residue was detected in the circulating lipophages. Our data suggest that lipophages are unlikely to be splenic origin.

INTRODUCTION

Among the experimental animals, guines pig has been demonstrated to show hemolytic anemia on feeding of cholesterol-rich diet (1-3). In a previous study lipid-laden monocytes have been detected in the blood films of anemic cholesterol-fed guines pigs (4). Such cells have been found in the enlarged spleens of cholesterol-fed guinea pigs (5). In an attempt to achieve insight into the reticulcendothelial origin of the circulating lipid-laden monocyte, we designed this experiment.

MATERIALS AND METHODS

Animals and diets:

English-Strain male guinea pigs were supplied by the animal unit of the Central Public Health Laboratory/

Baghdad. Their initial body weight ranged between 350-450gms. Normal guinea pig, chow was prepared and supplied by local supplier (Baghdad). To prepare 2% cholesterol-rich diet, ether-dissolved cholesterol was added to the normal guinea, pig chow

Experimental design

All the animals were fed normal guinea pig chow for two weeks to allow adaptation, and kept in singles per (15x15x45cm) cage. Throughout the experimental period the animals had free access to food and water. Water was supplemented with ascorbic acid (1 gm/L) to meet the animals daily requirement. Twenty guinea pigs were randomly allocated to two groups: Control group (C) kept on normal guinea pig chow for sixty days and high-cholesterol (HC) kept on a 2% cholesterol-rich diet for sixty days. By day 30 of the experiment, 4 animals from group C and 6 animals from group HC were sacrified. At the same proportion the rest of the animals were sacrified at day 60 of the experiment.

Haematological studies:

Peripheral blood films were prepared from all the animals at both day 30 and 60. Some of the films were fixed by formaline fume to be stained thereafter by Oil red-O and Perl's Prussian blue stains.

Blood cell morphology was studied after staining of the peripheral blood films with Wright-Giemsa stain.

The vaculated leukocytes in the peripheral blood films were characterized by two stains: the non specific estrase (NSE) stain (6) and Oil red-O stain. Using NSE stain identifies monocytes mainly, while neutrophils are only slightly stained. A leukocyte was considered as vacuolated when shows more than three cytoplasmic vacuoles.

RESULTS

At day 30, no significant difference was found in the size of spleen and liver of the animals of the HC group compared with those of the C group. By day 60, both

spleen and liver of the HC group showed, at least, a two-fold increase in size compared to the C group. The enlarged livers of the HC group revealed yellowish discoloration.

Peripheral blood films of the HC group demonstrated echinocytes at day 30 (figure 1), whereas no vacuolated leukocytes were shown. By day 60, such vaculated cells were detected in the peripheral blood of the HC group (Figure 2). The vacules were intracytoplasmic, variable in size and Oil red-O Positive (Figure 3). Upon staining with NSE, the vaculated cells were shown to be monocytes (figure 4). However, neutrophils revealed lower NSE-positivit, yet, non of them had intracytoplasmic vacuolation nor had Oil red-O positive reaction. The vacuolated monocytes were not positively stained with Perl's prussian blue.

DISCUSSION

Lipid-laden monocytes (Lipophages) were found in the peripheral blood of the HC group by day 60. The appearance of such lipophages has been an intriguing subject in the studies of atherogenesis and atherosclerotic plaque regression. In those studies a role was demonostrated for the blood-borne monocytes in scavanging lipid deposits from the arterial intima and migrating back into the circulation as foam cells[7, 8]. Interestingly, no detectable atheroma has been reported in the aortas of cholesterol fed guinea pigs[1, 4]. This suggests that there may be a different source of circulating lipophages.

The presence of lipid-laden macrophages in the enlarged spleen of cholesterol-fed guines pigs[5] and the cholesterol-rich erythrocytes (echinocytes)[1] in those animals has triggered the idea that splenic macrophage may be the origin of the circulating lipophages. Our results demonstrated echinocytes in the cholesterol-fed animals. Erythrophagocytosis and the "pitting process" of the spikes of the echinocytes most likely caused by the splenic macrophages[9, 10] Such erythrophagocytosis may

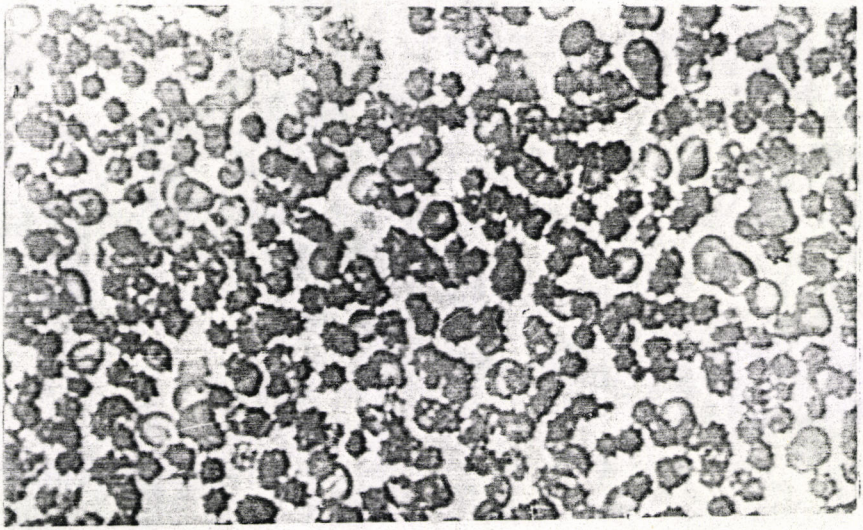

Figure 1: Peripheral blood smear from an animal kept on a cholesterol-rich diet at day 30. A large number of echinocytes is present (Wright-Giemsa stain x 1000).

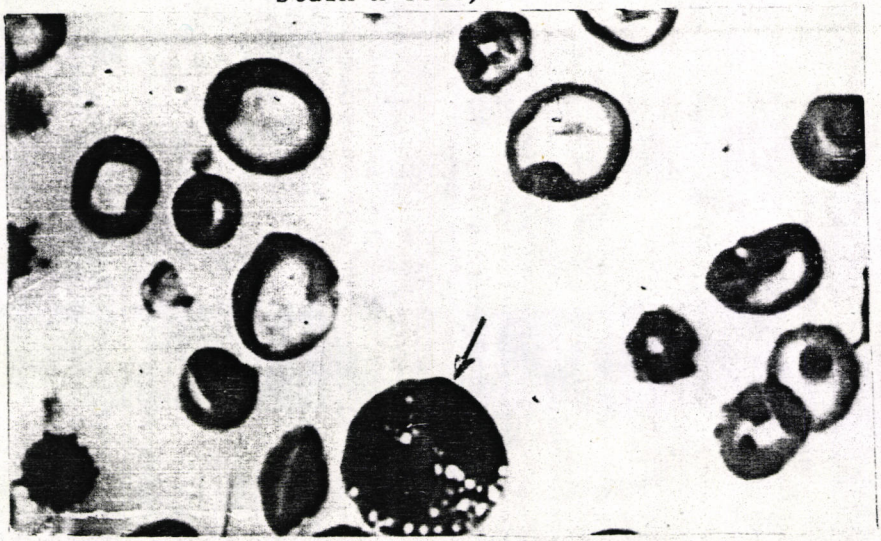

Figure 2: A Peripheral blood smear from the HC group at day 60 showing vaculated monocyte (arrow) (Wright-Giemsa stain x 1000).

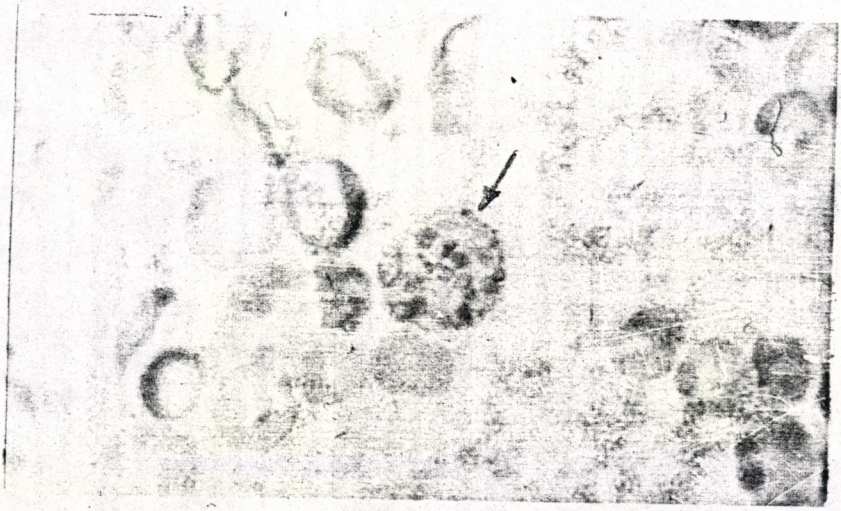

Figure 3: A peripheral blood smear from a cholesterol-fed animal at day 70. A leukocyte with oil red O-positive droplets (arrow). (oil red O stain x 1000)

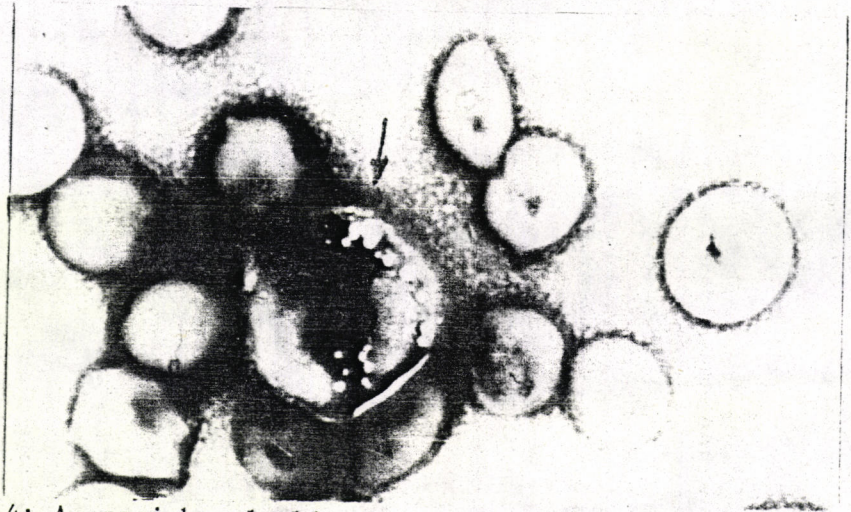

Figure 4: A peripheral blood smear from the HC group depicts NSE-positive vacuolated leukocyte (arrow). (NSE stain x 1000).

lead to accumulation of lipid inside the splenic macrophages. However, using Perl's Prussian blue stain revealed no hemosiderin residue in the circulating lipophages, a finding that makes it unlikely to depict those lipophages as migrating lipid-laden splenic macrophages to the circulation. Although no circulating lipophages have been reported in patients with familial or acquired hyperlipidemias, a role for lipid uptake by circulating moncyte has been postulated in hyperlipaemic rats[11, 12]. Excluding the splenic origin of the circulating lipophages in our study makes examining the lipid-uptake hypothesis worthwhile

REFERENCES

1. Ostwald, R. amd Shannon, A. (1964). Composition of tissue lipids and anemia of guinea pigs in response to dietary cholesterol. Biochemical J. 91: 146-154.

2. Yamanaka. W.; Winchell, H.S. and Ostwald R. (1967). Erythrokinetics in dietary hypercholesterolemia of guinea pigs. Am. J. Physiol. 213: 1278-1284.

3. Ostwald, R.; Yamanaka. W. and Light, M. (1970). The phospholipids of liver, plasma and red cells in normal and cholesterol-fed anemic guinea pigs. Proc. Soc. Exp. Biol. Med. 134:797.

4. Al-Shebeb, T.H.; Prohlich, J. and Magil, A.B. (1988). Glomerular disease in hypercholesterolemic guinea pigs: A pathogenetic study. Kidney Int. 33: 498-507.

5. Yamanaka, W.; Ostwald, J. and Magil, S.W. (1967). Histophathology of guniea pigs with cholesterol-induced anemia. Proc. Soc. Exp. Biol. Med. 124: 303-306.

6. Yam. LT Li Cy, and Crosby, W.H. (1971). Chtochemical identification of monocyte and granulocytes, Am. J. Clin. Path. 55: 283-290.

7. Gerrity, R.G, (1981). The role of moncyte in atherogenesis. II Migration of foam cells from atherosclerotic lesions Am. J. Pathol. 103: 191-200.

8. Faggiotto, A.; Ross, R. and Harker, L. (1984). Studies of hypercholesterolemia in the non-human primate. I changes that lead to fatty streak formation Arteriosclerosis 4: 323-340.

9. Babior, B.M. and Stossel, T.P. (1988). A pathophysiological Approach. Churchill Livingstone, New York, Edinburgh, London, Melborn. pp. 97-127.

10. Erslev, A.J. and Gabuzda, T.G. (1985). Survival disorders. In: Pathophysiology of Blood. 3rd Ed. W.B. Saunders Company. Philadelphia, London, Toronto, Mexico City. pp: 109-134.

11. Suziki, M. and O'Neal R.M. (1964). Accumulation of lipids in the leukocytes of rats fed atheroghenic diets J. Lipid Res. 5:624-627.

12. Suzuki, M. and O'neal, R.M. (1967). Circulating lipophages, serum lipids and atherosclerosis in rats. Arch. Pathol. 83: 169-174.

دراسة نسيجية كيمياوية حول خلايا الدم الملتهمة للدهون في خنازير غينيا المتغذية بغذاء عالي الكلسترول

طه حامد الشبيب(١) حكمت صاحب الناصر(٢)
و نجم عباس جابر(٣)
فرع الأمراض(١) فرع الصحة العامة(٣) فرع الطب و العلاج(٣)

كلية الطب البيطري - جامعة بغداد

الخلاصة

لقد اختيرت هذه الدراسة الفرضية القائلة بتأصيل خلايا الدم الملتهمة للدهون من خلايا الطحال العملاقة المحملة بالمواد الدهنية في خنازير غينيا المتغذية بغذاء غني بالكلسترول.

لقد وضعت حيوانات التجربة عشوائياً في مجموعتين الاولى ابقيت على غذاء عالي الكلسترول و الثانية على غذاء طبيعي واعتبرت الأخيرة مجموعة سيطرة. لقد استخدمت صبغات الرايت كمزا والأويل الحمراء و الاستريز غير المتخصصة بالاضافة الى صبغة البروسين الزرقاء في صبغ شرائح الدم في اليومين الثلاثين و الستين من التجربة.

لقد توضح وجود خلايا الدم البيضاء المتفجية في تلك الشرائح في اليوم الستين من التجربة ولقد ثبت بأن تلك الخلايا كانت محملة بالدهون وانها كانت من نوع احادية النواة، ولم يظهر أي أثر للهيموسيدرين فيها.

ولذلك فاننا نقترح استناداً لهذه النتائج بأن ليس من المحتمل أن تكون هذه الخلايا متأصلة من خلايا الطحال الالتهامية.