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Research Article

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Role of interleukin-1 beta in foam cell formation of THP-1 macrophages

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ABSTRACT

To investigate the effects of Interleukin-1beta(IL-1β) on foam cell formation of THP-1 macrophages (Mφs). Human THP-1 monocytes were differentiated into macrophages by phorbol esters in vitro, then the macrophages were incubated with the absence of IL-1β and oxidized low density lipoprotein (ox-LDL) (control group), 10ng/ml IL-1β (IL-1β group), 100ug/ml ox-LDL (ox-LDL group) or 10ng/ml IL-1β and 100ug/ml ox-LDL (IL-ox-LDL group) for 24h. High performance liquid chomatography was used for qualitative and quantitative analysis of intracellular cholesterol and cholesteryl esters. Both light microscope with red oil O staining technique and transmission electron microscope were employed to observe the morphology of treated and controlled THP-1 cells. A large number of intracellular red oil O stained granules and lipid vacuoles were observed in ox-LDL group and IL-ox-LDL group, the contents of total cholesterol and cholesteryl esters were significantly higher than those of control group (P<0.05), and the contents of cholesteryl esters were higher than 50% of total cholesterol in both groups. However, only few intracellular red oil O stained granules and lipid vacuoles were observed in control group and IL-1β group, there was no significant difference in the contents of total cholesterol and cholesteryl esters between control group and IL-1βgroup (P>0.05), and the contents of cholesteryl esters were less than 50% of total cholesterol in both groups. IL-1β alone cannot make THP-1 cells into foam cells, indicating that inflammatory cytokines were not independently atherogenic.

Key words: Interleukin-1β, oxidized low density lipoprotein, cholesterol, foam cell formation

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INTRODUCTION

Macrophage-derived foam cells are the key cellular elements in the early stages of atherosclerosis and play an important role in the development and progression of atherosclerosis though production of various active molecules. Such as cytokines and growth factors[1-4]. Macrophages take up oxidized LDL (ox-LDL) though the scavenger receptor pathways and become foam cells[5,6]. It has been shown that several cytokine and factors regulate scavenger receptor （SR）expression and/or activity[7]. GM-CSF is a positive regulator that increases the activity of the SR[8]. Tumor necrosis factor-α, TGF-β and GM-CSF are negative regulators.

Interleukin-1β(IL-1β), a member of the interleukin-1 superfamily, has a variety of important biological functions and plays an established role in vascular pathobiology[9-11]. Increased IL-1β mRNA expression has been demonstrated in atherosclerotic plaque[12,13]. Moreover, IL-1β upregulates the expression of various adhesion molecules (ie, intercellular adhesion molecule-1, vascular cell adhesion molecule-1) on the endothelial cell surface, promoting leukocyte adherence[14]. It also acts as a mitogen for smooth muscle cells and stimulates the release of other cytokines, for example, IL-6. Our clinical study has also reported elevated levels of IL-1β, total cholesterol (TC) and triglyceride (TG) in patients with coronary heart disease, and there are intrinsic correlation among these changes[15]. But the contribution to atherosclerotic development of IL-1β is not fully understood.

Thus, the present study was designed to examine whether or not the direct effects of IL-1β on foam cell formation, and it's histological changes.

EXPERIMENTAL SECTION

a.Materials

Human THP-1 monocytic leukemia cells were obtained from the cell band of the Institute of Biochemistry and Cell Biology, Shanghai Institute for Biological Sciences. RPMI-1640 medium was purchased from Gibcol Laboratories (Grand Island, NY). The following materials were acquired from various sources: fetal bovine serum (FBS) from Hangzhou Sijiqing Biological Engineering Materials Co, Ltd; bovine serum albumin (BSA) from Shanghai Pufei Bio-Technology Co, Ltd; phorbol 12-myristate 13-acetate (PMA) and IL-1β from Sigma; and low density lipoprotein (LDL) from Calbiocom. All other chemicals were of analytical grade.

b.Cell Cultuer

THP-1 monocytes were grown at 37℃ in a 5% CO_2 atmosphere to a density of 1×10^6/ml. Grown medium for THP-1 cells was RPMI1640 supplemented with 10% FBS, 100u/ml penicillin, and 100u/ml streptomycin, and 160nmol/L PMA for 48h to become fully differentiated macrophages before use in experiments. The cells further incubated in RPMI for 72h under the following conditions: (A) free of IL-1β and ox-LDL (control group); (B) IL-1β(10ng/ml, IL-1 group); (C) ox-LDL (50ug/ml, ox-LDL group); (D) IL-1β(10ng/ml) and ox-LDL (50ug/ml) (IL-ox-LDL group). Studies were performed 6 times.

c.Preparation of Ox-LDL

LDL (1000ug protein/ml) was incubated in F-10 medium (2ml) with added $CuSO_4$ (10umol/L) in 6-well dishes at 37℃ for 12h. Usually, 12ml of oxidized LDL was prepared at one time. The degree of oxidation was evaluated by measuring the concentration of thiobarbituric acid-reactive substances (TBARS). The average amount of TBARS generated for the 26 different oxidized LDL preparations used in this study was 74.8nmol MDA equivalented mg LDL protein 22.6 (mean $\pm$ SE).

d.Oil Red O Staining

The cells were washed 3 times with PBS, fixed with formaldehyde, and stained with 0.3% oil red O and hematoxylin. Cells were observed via light microscope and then photographed.

e.Transmission Electron Microscope

The cells were washed twice with PBS and removed with a rubber policeman. Cells were fixed in 2.5% gluteraldehyde, postfixed in 1% osmium teteroxide, and ultrathin sections were stained with uranyl acetate and lead citrate. A Phillips CN-120 microscope was used.

f.Lipid extraction

Lipids were extracted by using the method of Hara and Radin with modifications[16,17]. The cells were collected from the culture flasks into 0.9% NaCl (2 mL per 75 cm^2 flask) and homogenized on ice by sonication for 10s with a Sonifier 450 sonicator (Branson Ultrasonics, Danbury, USA) set to maximum power. The protein concentration of the cell lysate was determined by the method of Lowry *et al*[10]. To a volume of cell suspension known to contain 1 mg of protein was added 100 μg of cholesteryl heptadecanoate in chloroform as an internal standard. An equal volume of freshly prepared cold (-20^{o}C) KOH in ethanol (150 g/L) was then added and the cell lysate was repeatedly vortexed until clear with 6% trichloroacetic acid. An equal volume of 4:1 exane-isopropanol (*v*/*v*) was added and the mixture was vortexed for 5 min followed by centrifugation at 800×*g* and 15 ^{o}C for a further 5 min. The extraction procedure was repeated twice (a total of thee extraction procedures). The combined organic phase was transferred to clean tapered glass tubes and thoroughly dried in a vacuum freeze dryer at 65 ^{o}C. The tubes were allowed to cool to room temperature, 100 μL of the mixture of isopropyl alcohol-*n*-heptane-acetonitrile at 35:12:52 (*v*:*v*:*v*) was added, and the sample was solubilized by placing it in an ultrasound water bath for 5 min at room temperature. After centrifugation at 800×*g* for 5 min, 20 μL of the sample was introduced into the HPLC device.

g.Determination of cholesterol content of macrophage foam cells by HPLC

The cholesterol and cholesteryl ester content of cells were analyzed by HPLC as described previously[18,19]. HPLC was performed using a Waters device(Milford, USA) equipped with a model 1525 binary pump, a model 717 plus autosampler, a model 2487 dual absorbance detector, and a 4.6 mm×100 mm Gen-Pak FAX column (Waters). Waters' Breeze software was used to control the HPLC system. Cholesterol and cholesteryl esters were eluted isocratically at a flow rate of 0.5 mL/min and at a temperature of 4 ^{o}C using an eluent consisting of isopropanol-*n*-heptane-acetonitrile at the ratio of 35:12:52 (*v*:*v*:*v*) and detected by ultraviolet absorption at 206 nm.

h.Statistical analysis

All values were presented as mean ± SEM, and statistically significant differences ($p<0.05$) and extremely significant differences ($p<0.01$) among cadmium chloride-treated groups and the control were determined among

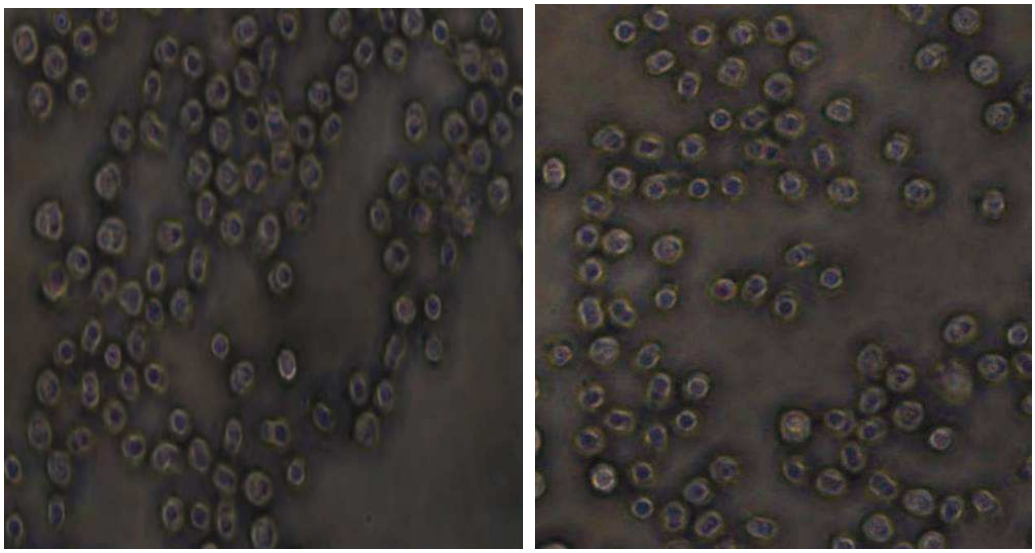
various groups by ANOVA and Tukey post-test using SPSS 12.0 statistical software

RESULTS AND DISCUSSION

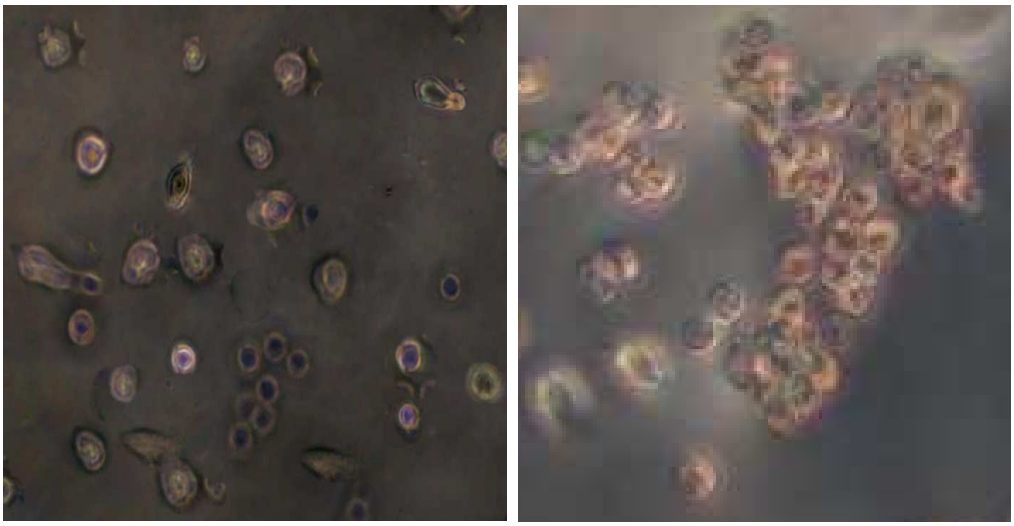
Characterization of THP-1 Monocyte-derived Macrophages/Foam Cells Induced by IL-1β or/and ox-LDL

The purpose of this series of experiments was to characterize by light and transmission electron microscopy IL-1β or/and ox-LDL loaded cultured macrophages during the differentiation of macrophages derived THP-1 monocytes. For this reason, the conditions for the various experiments were designed to match each group except for treated factors.

Lipid droplets inside the cells were stained with oil red O(red stained area), and examined with light microscopy (Fig 1): In control group(Fig1A) and IL-1βgroup(Fig1B), only few intracellular red stained area were observed, which suggests that the cytoplasm is filled with few lipid. In contrast, a large number of intracellular oil red O stained granules were observed in ox-LDL group(Fig1C) and IL-1β-ox-LDL groups(Fig1D), indicating that plenty of cholesteryl esters accumulated in the cytoplasm and the foam cell model was formed.



A **B**



C **D**

Figure 1. Light micrographs of THP-1 monocyte-derived macrophages stained with red oil O.

(a) Control macrophages after 24 h without ox-LDL or IL-1β. (b) Macrophages after 24h incubation with IL-1β. Many cell profiles are not stained, although a few show small,and distinct lipid inclusions(red stained area).(c) Macrophages after 24h of incubation with ox-LDL. A majority of cells in the field have many lipid inclusions. (d)

Macrophages after 24 h of incubation with ox-LDL and IL-1β. Almost all of the cells in the field have many lipid inclusions. Original magnification x 400.

By electron microscopy, lipid droplets are observed as electron-lucent vacuoles because lipids were extracted during dehydration with alcohol. Few lipid vacuoles were observed in control group(Fig2A) and IL-1βgroup(Fig2B). Whereas a bound of lipid vacuoles were found in ox-LDL group(Fig2C) and IL-ox-LDL group(Fig2D).

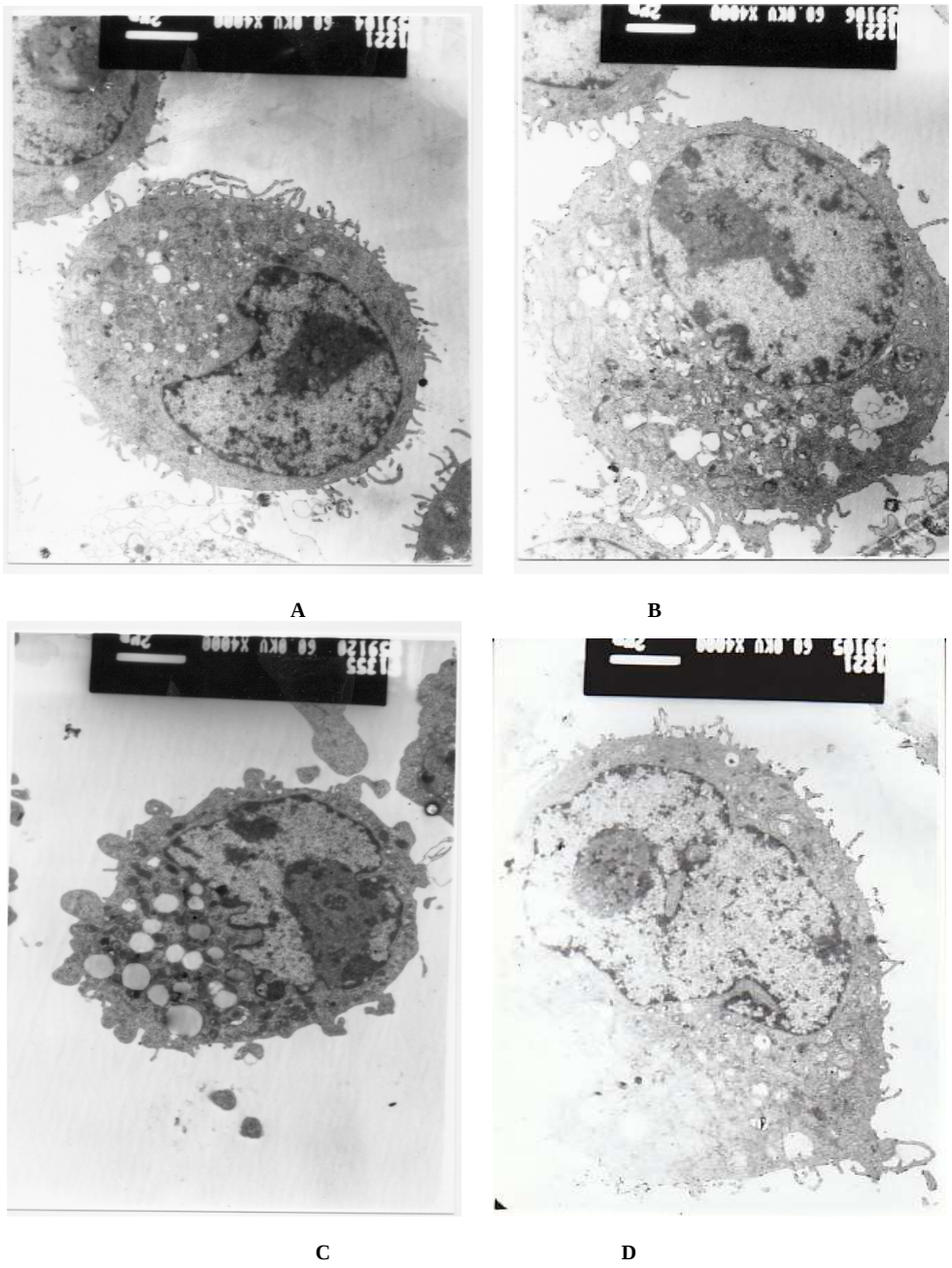


Figure 2. Transmission electron micrographs of THP-1 derived macrophages
(A) Control macrophage after 24 h incubation without ox-LDL. Cell cytoplasm has few of lipid vacuoles. (B) Macrophage after 24 h of incubation with IL-1β. Cells have a few of lipid vacuoles . (C) Macrophage after 24 h of incubation with ox-LDL. Cells have a lot of lipid vacuoles. (D) Macrophage after 24 h of incubation with ox-LDL and IL-1β. Cells have a bound of lipid vacuoles. Bars = 2μm.

Cholesterol Content in THP-1 Macrophages

Because cellular CE accumulation is known to parallel MΦ foam cell formation[20], we measured the effects of IL-1β on cellular CE and FC content. As shown in Fig3. The contents of total cholesterol and cholesteryl esters in ox-LDL group and IL-ox-LDL group were significantly higher than those of control group ($P<0.05$), and the contents of cholesteryl esters were higher than 50% of total cholesterol in both groups(64.1% vs 63.5%). However, there was no significant difference in the contents of total cholesterol and cholesteryl esters between control group

and IL-1βgroup (P>0.05), and the contents of cholesteryl esters were less than 50% of total cholesterol in both groups(32% vs 34%).

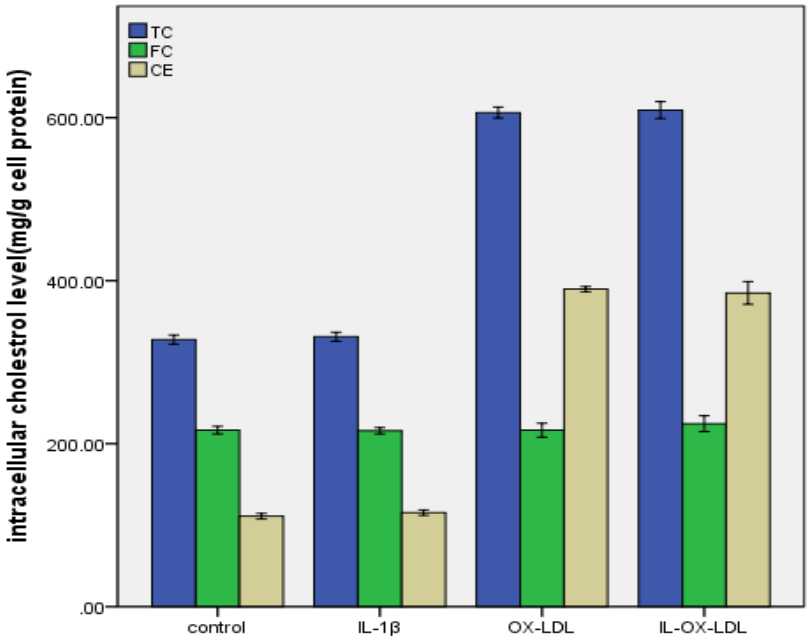


Figure 3. Accumulation of TC(blue bar) , FC (green bars) and CE (gray bars) in THP-1 macrophages. THP-1 macrophages were incubated for 24h with IL-1β or/and ox-LDL. The cells were harvested and the cellular cholesterol content was analyzed by HPLC as described in Materials and Methods

Values are means± SD for 6 dishes

Macrophage foam cells are a characteristic feature of atherosclerotic plaques. During foam cell formation, modified lipoproteins enters cells by receptor mediated uptake and excess neutral lipids are stored as lipid droplets, creating a typical foamy appearance[21]. The hallmark of these cells is their high lipid content, especially cholesteryl ester (>50% of total cellular cholesterol). Cytoplasmic lipid components increased significantly gather into lipid droplets, a foam-like changes stained with hematoxylin-eosin[22,23]. In our study, control group and IL-1β group, only few intracellular red stained area were observed, which suggests that the cytoplasm is filled with few lipid. In contrast, a large number of intracellular oil red O stained granules were observed in ox-LDL group and IL-ox-LDL groups, indicating that plenty of cholesteryl esters accumulated in the cytoplasm and the foam cell model was formed. Similar results were obtained using electron micrographs. Furthermore, the contents of total cholesterol and cholesteryl esters in ox-LDL group and IL-ox-LDL group were significantly higher than those of control group, and the rates of cholesteryl esters occupied total cholesterol are 64.1% and 63.5% respectively. However, there was no significant difference in the contents of total cholesterol and cholesteryl esters between control group and IL-1β group, and the rates of cholesteryl esters and total cholesterol were less than 50%(32% vs 34%). Based on the morphological and chemical characteristics of foam cells, These results suggest that foam cells are formed when THP-1 cells are incubated with ox-LDL, while IL-1β alone can not convert THP-1 cells into foam cells.

Despite IL-1β alone can not make the THP-1 macrophages into foam cells, which does not abolish the correlation between IL-1β and atherosclerosis, which suggests the involvement of additional regulatory mechanisms. In fact, Many pathways may be involved in lipid accumulation and foam cell (cells) formation[24,25]. ox-LDL are the modified lipoprotein usually considered causative during foam cell formation *in vivo*[26], but excessive uptake of other lipoproteins can also give rise to intracellular lipid accumulation and give macrophages a foamy appearance[27-29]. Xiong Z. Ruan et al [30] demonstated that imflammatory cytokines can modify cholesterol-mediated LDL receptor regulation in mesangial cells, permitting unregulated intracellular accumulation of unmodified LDL and causing foam cell formation. IL-1β may share a previously unknown effect on macrophage foam cells, resulting in retention of neutral lipids by a combination of decreased lipid efflux, decreased β-oxidation substrate availability, and stimulated fatty acid esterification[31]. But our data suggests IL-1β only induced a slight and insignificant increase in intracellular lipid accumulation in THP-1 macrophages. The reasons may be that (a) ox-LDL contains a number of highly pro-inflammatory and cytotoxic substances making it difficult to differentiate between the direct effects of these components and the effect of lipid loading in itself on macrophage function; (b)

IL-1β also enhanced lipid accumulation, but to a much lesser extent than ox-LDL. We hypothesize that ox-LDL mask the effects of IL-1β because IL-1β and ox-LDL use similar mechanism to induce foam cells formation.

CONCLUSION

IL-1β alone cannot make THP-1 cells into foam cells, indicating that inflammatory cytokines, such as IL-1β, were not independently atherogenic, which affect foam cell formation by regulating intracellular lipid metabolism. That is to say, intracellular dyslipidemia is necessary for the macrophages to turn into foam cells.

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REFERENCES

[1]Ross R. *Nature*.**1993**;362:801-809.
[2]Clinton SK, Libby P. *Arch Pathol Lab Med*. **1992**;116(12):1292-300.
[3]Hansson GK. *N Engl J Med.* **2005**; 352**:** 1685 – 1695.
[4]Stoll G, Bendszus M. *Stroke***. 2006**;37(7):1923-1932.
[5]Blasi C. *Atherosclerosis*.**2008**; 201 (1): 17–32.
[6]Poli G, Sottero B, Gargiulo S, Leonarduzzi G. *Mol. Aspects Med*. **2009**;30 (3):180–189.
[7] Kleemann R, Zadelaar S, Kooistra T. *Cardiovasc Res.* **2008**;79(3):360-376.
[8]Clinton SK, Underwood R, Hayes L, Sherman ML,Kufe DW,Libby P..*Am J Pathol.* **1992**;140(2):301-316.
[9]Dinarello CA. *Blood.* **1996**;87**:** 2095 – 2147.
[10]Dinarello CA. Blood. **2011**;117(14):3720-3732.
[11]Dinarello CA. *Eur J Immunol.* **2011** May;41(5):1203-1217.
[12]Dewberry R, Holden H, Crossman D, Francis S. *Arterioscler Thomb Vasc Biol* **2000**;20**:** 2394 – 2400.
[13]Olofsson PS, Sheikine Y, Jatta K, Ghaderi M, Samnegard A, Eriksson P,Sirsjo A. *Circ J*. **2009** Aug; 73(8): 1531-1536.
[14]Zerr M, Hechler B, Freund M, Magnenat S, Lanois I, Cazenave JP, Leon C, Gachet C. *Circulation*. **2011** 31;123(21):2404-2413.
[15]Wang Y, Cheng B, Feng YB. *Journal of Clinical Cardiology*.**2003**;19 (6):344-346.
[16]Hara A, N. S. Radin. *Anal Biochem.* **1978**;90: 420-426.
[17]Cullen P, Fobker M, Tegelkamp K, Meyer K, Kannenberg F, Cignarella A, Benninghoven A, Assmann G.*J Lipid Res. **1997**.38:*401-409.
[18] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. J *Biol Chem.* **1951**;193: 265-275.
[19]Contreras JA,Castro M,Bocos C, Herrera E,Lasuncion MA. *J Lipid R*es. **1992**;33(6):931-936**.**
[20]Brown MS, Goldstein JL, Krieger M, Ho YK,Anderson RG*. J Cell Biol.* **1979**;82(3):597-613.
[21]McLaren JE, Michael DR, Ashlin TG, Ramji DP. *Prog Lipid Res*. **2011** 13;50(4):331-347.
[22] Sano J,Shirakura S,Oda S, Hara T,Ishihara T. *Pathol Int.***2004**;54(12):904—913.
[23]Fogellman AM,Schechter I,Seager J., Hokom M, Child JS, Edwards PA. *Proc Natl Acad Sci* USA.**1980**; 77(4): 2214-2218.
[24] Ghosh S. *Expert Rev Cardiovasc Ther*. **2011** ;9(3):329-340.
[25] Ley K, Miller YI, Hedrick CC. *Arterioscler Thomb Vasc Biol*. **2011**;31(7):1506-1516.
[26]Maor I,Aviram M. *J Lipid Res*. **1994**;35(5):803-819.
[27]Persson J, Degerman E, Nilsson J, Lindholm MW. *Biochem Biophys Res Commun* **2007**;363**:**1020-1026.
[28]Boström P, Magnusson B, Svensson PA, Wiklund O, Borén J, Carlsson LM, Ståhlman M, Olofsson SO, Hultén LM. *Arterioscler Thomb Vasc Biol*. **2006**;26(8):1871-1876.
[29]Olofsson SO, Boström P, Andersson L, Rutberg M, Levin M, Perman J, Borén J et al.*Curr Opin Lipidol.* **2008**;19(5):441-447.
[30]Ruan XZ, Varghese Z, Powis SH, Moorhead JF.*Kidney International*.**2001**;60:1716—1725.
[31]Persson J, Nilsson , Lindholm MW. *BMC Immunology.* **2008** Nov 25;9:70