Enhanced Cholesterol Esterification in Monocyte-Derived Macrophages from Diabetic But Not Hypertriglyceridemic Men

Kazuko HIRAMATSU^{1,*} and Paul K. NAKANE²

¹*Department of Internal Medicine, Tokai University School of Medicine, Isehara 259-11, Japan*

²*Department of Anatomy, Nagasaki University School of Medicine, Nagasaki 852, Japan*

(Received November 15, 1990)

Summary To test whether monocyte-derived macrophages obtained from diabetic patients with hypertriglyceridemia have an altered manner of lipid accumulation, we separated monocytes from 7 healthy control subjects, 9 diabetic normolipidemic patients, 9 diabetic hypertriglyceridemic patients, and 7 nondiabetic hypertriglyceridemic patients. The monocytes of each group were modulated to macrophages under two different culture systems using either autologous serum or heterologous normal non-diabetic non-hypertriglyceridemic serum in the culture medium.

The cells transformed better in the healthy serum system. In this system, the [¹⁴C]oleate incorporation into cholesterol oleate by macrophages derived from the diabetic group (0.30 ± 0.11 nmol/mg protein/12 h (mean $\pm$ SD), $p < 0.05$) and diabetic-hypertriglyceridemic group (0.40 ± 0.12 nmol/mg protein/12 h, $p < 0.005$) was significantly higher than that by macrophages derived from the hypertriglyceridemic group (0.21 ± 0.16 nmol/mg protein/12 h) and healthy group (0.22 ± 0.02 nmol/mg protein/12 h). With the autologous serum system, similar results were obtained, although there was more fluctuation of the values within a group. When the degradation of labeled lipoproteins in the macrophages was determined concomitantly, the macrophages from all four groups degraded ¹²⁵I-acetyl LDL equally; however the rates of ¹²⁵I-labeled "hypertriglyceridemic" VLDL degradation were significantly lower by macrophages from the diabetic group (8.9 ± 3.5 μ g/mg protein/12 h, $p < 0.05$) and the diabetic-hypertriglyceridemic group (8.1 ± 1.2 μ g/mg protein/12 h, $p < 0.01$) than by those from healthy group (12.4 ± 2.6 μ g/mg protein/12 h) and hypertriglyceridemic group (11.4 ± 3.7 μ g/mg protein/12 h).

*To whom correspondence should be addressed.

grown in the healthy serum culture system.

Macrophages from diabetic patients may have accelerated cholesterol esterification because of the lowered VLDL influx. The increase was strongly linked with the diabetic condition and was not unique to the diabetic-hypertriglyceridemic patients. This trend may accelerate atherosclerosis in diabetic hypertriglyceridemia when an atherogenic factor specific to hypertriglyceridemia coexists.

Key Words: human monocyte, macrophage, diabetes, atherosclerosis, hypertriglyceridemia

The incidence of atherosclerosis is much higher in patients with diabetes and hypertriglyceridemia than in patients with either diabetes or hypertriglyceridemia [1, 2]. The reason for this is not clear; however, it has been speculated that the concomitant presence of atherogenic factors associated with diabetes and those associated with hypertriglyceridemia may be responsible for this high incidence rather than the presence of a unique atherogenic factor in the diabetic-hypertriglyceridemic patients. For example, glycosylated low-density lipoprotein found in the plasma of diabetics [3] and abnormal very-low-density lipoprotein (VLDL) found in the plasma of hypertriglyceridemic patients [4] are thought to be responsible for the accelerated foam cell formation. It has been assumed that the macrophages of the diabetic-hypertriglyceridemic patients, being exposed to these two abnormal lipids, accumulate lipids more aggressively, thus resulting in accelerated atherosclerosis. Although the presence of these as well as other humoral atherogenic factors and their effect on cells associated with the atherosclerotic plaques have been considered as the prime reason for the onset of atherosclerosis, the various types of cells associated with the atherosclerotic plaque must also be considered. In our previous studies, we found that freshly prepared monocytes from hypertriglyceridemic patients had an accelerated rate of superoxide synthesis [5]. In this study, we concentrated on monocyte-derived macrophages and asked whether or not the ability of these cells to synthesize cholesterol ester differs among the cells taken from the diabetic-hypertriglyceridemic, diabetic, hypertriglyceridemic, and healthy individuals, since these cells have been considered as the precursor to the form cells found in the atherosclerotic plaques [6]. Our observations suggest that the diabetic-hypertriglyceridemic patients are more prone to develop atherosclerosis because they have an elevated rate of cholesterol esterification and atherogenic factors specific to hypertriglyceridemia, such as increased superoxide production by monocytes, and that these conditions act synergistically to accelerate atherosclerosis.

MATERIALS AND METHODS

Subjects. Four groups of subjects, all men, were studied after informed consent had been obtained: 7 individuals who had neither diabetes mellitus nor hypertriglyceridemia (healthy group); 9 individuals who had diabetes mellitus but not hypertriglyceridemia (diabetic group); 9 individuals who had hypertriglyceridemia but not diabetes mellitus (hypertriglyceridemic group); and 7 individuals who had both diabetes mellitus and hypertriglyceridemia (diabetic-hypertriglyceridemic group). Patients with alcoholism, uremia, or hypothyroidism were excluded. All diabetic patients were non-insulin-dependent diabetes mellitus (NIDDM), having been diagnosed as such based on the criteria of the National Diabetes Data Group for a 75-g oral glucose tolerance test [7]. Blood glucose of diabetic patients was controlled with a stable treatment regimen for 4 months before the experiments, and glucose levels were stable at the time of blood withdrawal. Nine of the diabetic groups were being treated with insulin, 8 with oral hypoglycemic agents, and 1 with diet alone. Hypertriglyceridemia was diagnosed when the fasting plasma triglyceride levels were >150 mg/dl on three occasions. The 75-g oral glucose tolerance test was also performed on all patients with hypertriglyceridemia. Of those with diabetes and hypertriglyceridemia, five were being treated with insulin; 5, with oral hypoglycemic agents; and 3, by diet alone. Of those with hypertriglyceridemia, three were being treated with clonofibrate. At the time of blood collection, none of the subjects had evidence of acute infection or other inflammatory processes.

Preparation of monocytes. The experiments were performed on eleven separate days. On each day, 6 to 7 subjects were examined. Following an overnight fast, 110 ml blood was withdrawn from an antecubital vein into three syringes; 60 ml blood was collected into syringes containing heparin to give a final concentration of 10 units/ml for the separation of mononuclear cells, 40 ml blood was collected without any anticoagulant to obtain serum, and 10 ml blood was collected into a syringe containing EDTA to give a final concentration of 1 mg/ml for plasma lipid analysis. Heparinized blood was layered over a Conray-Ficoll gradient ($d=1.077$) and centrifuged for 30 min at $500\times g$ at 23°C . The mixed mononuclear cell band was removed into siliconized plastic tubes by aspiration with a siliconized glass Pasteur pipette. Cells were washed twice with RPMI 1640 medium, and the cell numbers of monocytes and lymphocytes in mononuclear cell population were counted in a Coulter Channelyzer (Hialeah, FL). Autologous serum was diluted into RPMI 1640 medium to give a final concentration of 20%, and the medium was sterilized by passage through a $0.22\text{-}\mu\text{m}$ filter (Millipore Inc., Yamagata). Mononuclear cells were dispersed into the medium at a concentration of 1×10^6 monocytes/ml, and 1 ml of cell suspension was seeded into each 35-mm well of a culture dish (Falcon and Primaria, Becton Dickinson Co., Lincoln Park, NJ). The residual monocytes were also plated into 16-mm wells. After a 2-h

incubation at 37°C in a humidified atmosphere of 95% air and 5% CO₂ to allow monocytes to adhere to the bottom of the well, non-adherent cells were removed by aspiration. After washing twice with warmed RPMI 1640 medium, monocytes were cultured as follows. All procedures were performed under sterile conditions.

Culture for transformation of monocytes to macrophages. Monocytes from each subjects were cultured for 10 days in two systems. The first used 1.5 ml of RPMI 1640 medium containing 20% autologous serum. The other used medium containing 20% heterologous healthy serum from a single source, a 22-year-old healthy man (blood type A). Medium was changed every 3 days. At the end of culture, the viability of the cells was determined by the trypan blue exclusion test, and over 85% of the cells were alive. The character of the cells was checked by alpha-naphthyl-butyrate staining, and the percent of monocyte-derived macrophages was estimated to be approximately 80%.

Lipoprotein separation. Two hundred milliliters of blood was drawn from a normolipidemic healthy man after an overnight fast into tubes containing EDTA (1 mg/ml). Low-density lipoprotein ($d=1.019-1.063$) was separated from the plasma by ultracentrifugation [8]. For the separation of hypertriglyceridemic very-low-density lipoprotein ($d<1.006$), 200 ml blood was drawn after an overnight fast from hypertriglyceridemic patients having a plasma triglyceride level of 1,100 mg/dl, and the separated plasma was subjected to ultracentrifugation. VLDL was separated after the removal of chylomicrons.

Acetylation of LDL was performed as described [9]. Both acetyl LDL and hypertriglyceridemic (HTG) VLDL were labeled with ¹²⁵I by the iodine monochloride method as modified for lipoproteins [10].

[¹⁴C]Oleate incorporation into ¹⁴C cholesteryl ester. On the 10th day of culture, culture medium was removed from 35-mm well, and the macrophages were washed twice with RPMI 1640 medium. One milliliter of plain RPMI 1640 medium was added and cells were cultured for 12 h in the presence of both 20 nmol [¹⁴C]oleate-albumin (7,400 dpm/nmol) and 50 µg of acetylated LDL. After incubation, the medium was removed and the cells were washed twice with phosphate-buffered saline. Macrophages were completely dissolved in 1 ml of 0.1 N NaOH after a 2-h incubation on a 37°C shaking water bath, and 0.9 ml of the sample was used for lipid extraction. The lipid extraction was performed with chloroform-methanol (2:1, v/v) [11]. Cholesteryl [¹⁴C]oleate was isolated by thin-layer chromatography after development in a solvent system of *n*-hexane-diethyl ether-formic acid (90:10:1, v/v/v). Lipids were visualized with I₂ vapor, and the spots that comigrated with a cholesteryl oleate standard were marked and scraped into scintillation vials after the total disappearance of color. Scintillation fluid (Aquasol 2, NEW Research Products, Boston, MA) was added, and the samples were counted in a liquid scintillation counter. Correction for procedural losses was made by addition of [³H]cholesteryl oleate to the chloroform-methanol extraction mixture as an internal standard. The remaining 0.1 ml of dissolved cells was used for the measurement of cell protein [12]. Each experiment was performed

in triplicate.

¹²⁵I-acetyl LDL and ¹²⁵I-labeled hypertriglyceridemic VLDL degradation by macrophages. Macrophages cultured in heterologous healthy serum in 16-mm wells were used for the assay of total ¹²⁵I-acetyl LDL degradation and total ¹²⁵I-labeled hypertriglyceridemic VLDL degradation. The amount of each added lipoprotein in terms of protein was 5 µg/ml for ¹²⁵I-acetyl LDL and 50 µg/ml for ¹²⁵I-labeled hypertriglyceridemic VLDL. The degradation assay was performed as described previously [13]. All samples were run in triplicate.

Miscellaneous analyses. Fasting plasma glucose, total cholesterol, triglyceride, and hemoglobin A_{1c} were measured in the Tokai University Hospital Central Laboratory by commonly used procedures.

Statistical method. Comparisons between the groups were made by Student's *t*-test [14]. All values were expressed as means ± SD.

RESULTS

The diabetic and diabetic-hypertriglyceridemic groups had higher fasting plasma glucose levels accompanied by higher levels of hemoglobin A_{1c} than the healthy and hypertriglyceridemic groups (Table 1). The mean plasma triglyceride levels in the hypertriglyceridemic and diabetic hypertriglyceridemic groups were approximately threefold higher than those in the healthy and diabetic groups. The amount of triglyceride was approximately the same between the hypertriglyceridemic and diabetic hypertriglyceridemic groups.

A comparison of the cellular protein of macrophages obtained from 35-mm wells under the two different culture conditions is shown in Fig. 1. The protein amount of 0.075 mg per dish was employed as a cut-off point, as this was the minimum limit of the reliable range of our method used for protein measurement. The majority of the macrophage samples grew better when cultured in the normal serum system than in the autologous serum one. The number of samples in which the yield of protein was over 0.075 mg per dish after 10 days of culture was also greater for cultures with the normal serum system than for those with the

Table 1. Plasma glucose, HbA_{1c} and lipid concentration in healthy, diabetic, diabetic-hypertriglyceridemic, and hypertriglyceridemic subjects.

Group	<i>n</i>	Glucose (mg/dl)	HbA _{1c} (%)	Triglyceride (mg/dl)	Cholesterol (mg/dl)
Healthy	7	95 ± 5 (87-97)	ND	114 ± 32 (68-145)	207 ± 22(170-218)
Diabetic	9	139 ± 54(67-270)	8.1 ± 1.9	75 ± 33 (29-136)	194 ± 27(139-220)
Hypertriglyceridemic	9	102 ± 10(83-110)	ND	357 ± 251(192-861)	223 ± 49(182-299)
Diabetic-hypertriglyceridemic	7	176 ± 62(121-230)	8.5 ± 1.4	309 ± 142(204-612)	233 ± 23(210-284)

Values are means ± SD with ranges in parentheses. Normal range of hemoglobin A_{1c} (HbA_{1c}) is 5.2-6.7%. ND, not determined.

Fig. 1. Comparison of protein content of macrophages cultured with autologous serum or with heterologous healthy serum. Protein content of macrophages cultured in 35-mm wells in the autologous serum system are plotted on the left and those in the normal serum system are on the right. The black dots depict samples in which protein content decreased in the healthy serum system compared with the autologous serum system.

Table 2. Numbers of samples in which monocytes matured to macrophages.

Group	Incubated with healthy serum		Incubated with autologous serum	
	Number	Protein per 35-mm dish (mg)	Number	Protein per 35-mm dish (mg)
Healthy	5/7	0.36 ± 0.25	2/7	0.38 ± 0.07
Diabetic	8/9	0.38 ± 0.33	8/9*	0.39 ± 0.25
Hypertriglyceridemic	8/9	0.49 ± 0.20	5/9	0.39 ± 0.29
Diabetic-hypertriglyceridemic	7/7	0.52 ± 0.27	5/7	0.27 ± 0.18

* $p < 0.02$: chi square test between non-diabetic and diabetic groups.

autologous serum culture system except in the diabetic group. In the autologous serum culture system, 8 of 9 reached over 0.075 mg in the diabetic group, significantly higher than the 2 out of 7 in the non-diabetic group (Table 2). No significant difference was observed in the number of samples that had over 0.075 mg protein per dish among the non-diabetic, hypertriglyceridemic, and diabetic-hypertriglyceridemic groups. When the mean value of macrophage protein was calculated employing only these data over 0.075 mg, macrophage protein did not differ among the four groups (Table 2). In comparing macrophage protein between the two culture systems, the amount of protein was similar in the non-diabetic and diabetic groups and higher in the normal serum culture system than with autologous system in the hypertriglyceridemic and diabetic-hypertriglyceridemic groups.

[^{14}C]Oleate incorporation into cholesteryl ester by macrophages was measured in the presence of acetyl LDL as shown in Table 3. In the normal serum

Table 3. [^{14}C]Oleate incorporation by macrophage into cholesteryl oleate per cellular protein.

Group	Incubated with normal serum	Incubated with autologous serum
Healthy	0.215 ± 0.019 (5)	0.287 ± 0.054 (2)
Diabetic	0.314 ± 0.110 (8)*	0.852 ± 0.568 (7)
Hypertriglyceridemic	0.212 ± 0.164 (8)	0.175 ± 0.127 (5)****
Diabetic-hypertriglyceridemic	0.403 ± 0.115 (7)**,**	0.471 ± 0.361 (5)

The units for all values are nmol/mg protein/12 h. Sample number is indicated in parentheses. * $p < 0.05$ vs. healthy; ** $p < 0.005$ vs. healthy; *** $p < 0.02$ vs. hypertriglyceridemic; **** $p < 0.02$ vs. corresponding diabetic.

Table 4. ^{125}I -acetyl LDL degradation by macrophages.

Group	N	Degradation
Healthy	4	18.2 ± 6.1
Diabetic	3	18.6 ± 8.9
Hypertriglyceridemic	3	15.7 ± 8.2
Diabetic-hypertriglyceridemic	3	14.4 ± 2.3

All values are expressed as $\mu\text{g}/\text{mg}$ cellular protein/12 h.

culture system, the amount of [^{14}C]oleate incorporation into cholesteryl ester was highest in the diabetic-hypertriglyceridemic group (0.403 ± 0.115 nmol/mg cellular protein/12 h), followed by the diabetic (0.314 ± 0.110), non-diabetic (0.215 ± 0.019), and hypertriglyceridemic group (0.212 ± 0.164). Both the diabetic and diabetic-hypertriglyceridemic groups were significantly higher than the non-diabetic group. [^{14}C]Oleate incorporation into cholesteryl ester in the diabetic-hypertriglyceridemic group was also significantly higher than that in the hypertriglyceridemic group.

In the autologous serum incubation system, the amount of [^{14}C]oleate incorporation into cholesteryl ester by macrophages fluctuated greatly and showed a large variation. However macrophages from the diabetic group showed significantly higher [^{14}C]oleate incorporation into cholesteryl ester compared with the cells from the hypertriglyceridemic group. A comparison of [^{14}C]oleate incorporation into cholesteryl ester by macrophages between the two different incubation systems showed no difference except in the diabetic group where incorporation was significantly greater in the autologous culture system than in the normal serum culture system.

To assess the receptor activity responsible for the influx of lipid into the macrophages, we measured the degree of degradation of ^{125}I -acetyl LDL and of ^{125}I -HTG-VLDL by macrophages. The amount of ^{125}I -acetyl LDL degradation was similar among the four groups (Table 4). The degradation of ^{125}I -HTG-VLDL was significantly decreased in the diabetic group (8.9 ± 3.5 $\mu\text{g}/\text{mg}$ cellular protein/12 h, $p < 0.01$) and in the diabetic-hypertriglyceridemic group (8.1 ± 1.2 $\mu\text{g}/\text{mg}$ cellular protein/12 h, $p < 0.05$) compared with that in the non-diabetic group (12.4 ± 2.6 $\mu\text{g}/\text{mg}$ cellular protein/12 h), as shown in Table 5.

Table 5. ^{125}I -HTG-VLDL degradation by macrophages.

Group	N	Degradation
Healthy	5	12.4 ± 2.6
Diabetic	8	$8.9 \pm 3.5^*$
Hypertriglyceridemic	5	11.4 ± 3.7
Diabetic-hypertriglyceridemic	5	$8.1 \pm 1.2^{**}$

All values are expressed as $\mu\text{g}/\text{mg}$ cellular protein/12 h. $^*p < 0.05$ vs. non-diabetic;

$^{**}p < 0.01$ vs. non-diabetic.

DISCUSSION

There were two major findings in our study. First, monocyte-derived macrophages of diabetic-hypertriglyceridemic and diabetic patients have an elevated ability for cholesterol esterification compared with the macrophages of hypertriglyceridemic and healthy individuals. Second, monocyte-derived macrophages of diabetic-hypertriglyceridemic and diabetic patients have less ability to degrade HTG-VLDL than macrophages of hypertriglyceridemic and healthy individuals.

The rate of cholesterol esterification by macrophages was determined in the presence of acetyl LDL and ^{14}C -labeled free fatty acid-albumin complex. With this method, acetyl LDL and free fatty acid-albumin complexes were taken up by the macrophages. The acetyl LDL and complex would then enter lysosomes to be digested. Products of this process, such as cholesterol and free fatty acid, would then serve as substrates for the synthesis of new cholesteryl ester. Thus, the observed increased rate of cholesterol esterification with the monocyte-derived macrophages of diabetic-hypertriglyceridemic and diabetic patients may have resulted for various reasons, such as increased substrate pool or increased anabolic enzymatic activities.

The substrate pool may be increased by an elevated rate of uptake of acetyl LDL and/or free fatty acid-albumin complex, and/or by an increased size of the pool of endogenous cholesterol. In the present study, we measured only the total amount of degradation, and the degradation rate of ^{125}I -labeled acetyl LDL was the same in the diabetic cells as in the hypertriglyceridemic and normal macrophages. Thus it may be assumed that the rate of uptake of acetyl LDL was the same among the four groups. As for a possible increase in the rate of uptake of free fatty acid-albumin complex, our current study has provided no new information. However, in this regard, the findings by Vlassara *et al.* [15] that peritoneal macrophages obtained from the insulin-deficient diabetic mouse showed increased receptor activity for the advanced glycosylated end product-albumin complex is of interest. Whether or not the advanced glycosylated end product-albumin complex shares the receptor with the free fatty acid-albumin complex and such receptors are increased on the surface of the diabetic-hypertriglyceridemic and diabetic macrophages has not been demonstrated. If this is true, then the influx of free fatty

acid-albumin complex into the macrophages may have been elevated by this route and thus have increased the substrate pool.

Glycosylated LDL, which is found in diabetic sera and is taken up via the glycosylated LDL receptor of macrophages [3], was demonstrated to enhance cholesteryl ester synthesis; and this synthesis could not be down-regulated even when the macrophages accumulated an excessive amount of cholesteryl ester. In our study, it is unlikely that the elevated rate of cholesterol esterification resulted from the uptake of glycosylated LDL by the diabetic-hypertriglyceridemic and diabetic macrophages since these cells were maintained with normal serum that should have been free of glycosylated LDL. On the other hand, in the diabetic-hypertriglyceridemic and diabetic macrophages that were maintained with autologous serum, the glycosylated LDL may have represented a portion of the intracellular cholesterol pool since the rate of cholesterol esterification of these cells was even greater than that of those maintained in normal serum-containing medium.

The decreased rate of VLDL degradation by the diabetic-hypertriglyceridemic and diabetic macrophages may be responsible for the increased rate of cholesterol esterification by these cells. The following two studies had been performed employing the mouse macrophages. Angelin [16] has reported that VLDL suppresses 3-hydroxy-3-methylglutaryl- (HMG-) CoA reductase activity, a key enzyme for cholesterol synthesis, in mouse peritoneal macrophages. Similar to this observation, Kraemer [17] found that peritoneal macrophages from streptozocin-induced diabetic-hypertriglyceridemic mice had enhanced HMG-CoA reductase activity, and the increase was secondary to the reduction in the number of VLDL receptors but not related to acetyl-LDL uptake. These reports, when considered together, raise the possibility that a reduction in the rate of VLDL uptake may attenuate the inhibitory activity of VLDL on HMG-CoA reductase, resulting in more cholesterol synthesis and an enlarged endogenous cholesterol pool. Hence, if the mechanism elucidated for the mouse macrophage also works in human macrophages, the reduced rate of catabolism of HTG-VLDL in monocyte-derived macrophages from diabetic patients may have increased the activity of HMG-CoA reductase and elevated the rate of endogenous cholesterol synthesis. If the amount of cholesterol synthesized by the endogenous pathway exceeds the amount of cholesterol normally derived from VLDL, the substrate pool would be enlarged, and this should lead to an increased rate of cholesteryl ester synthesis. The current investigation was not designed to prove this possibility, and the task is left to future investigations.

Another question asked in this investigation was whether the rate of cholesteryl ester synthesis by macrophages was intrinsic to the cells. Prior to the start of the experiments, it was necessary to establish conditions for the macrophage cell culture. Human macrophages are usually derived from circulating monocytes in culture medium containing autologous serum, and the use of such a system does not discriminate between effects due to the cells and those due to the serum. In

preliminary experiments, we found that human circulating monocytes were difficult to maintain and showed poor transformation into macrophages in standard cell culture medium with fetal calf serum (data not shown). Consequently, isolated monocytes were cultured in media containing either autologous serum or heterologous serum from a single healthy male donor. Although in the beginning, the blood type and sex were matched between cells and serum, we later found that blood type had no effect on the growth of monocytes (data not shown). Nevertheless, one batch of serum from blood of a type A healthy man was used throughout the experiment in order to minimize variables.

Among those microphages that were derived from monocytes in the normal serum system, the rate of degradation of ^{125}I -HTG-VLDL and the rate of cholesterol esterification depended upon the source of the monocytes. This demonstrated that by the time monocytes were transferred to the *in vitro* system and before the cells had changed to macrophages, the potential of the macrophages to degrade HTG-VLDL and to synthesize cholesteryl ester may have already been determined.

What determines this potential is both interesting and important to an understanding of atherogenesis. Assuming that it is epigenetic, at some stage of myelopoiesis, the cells either acquired this potential or only those which have the potential matured. Knowing that all cells in the body are bathed in the milieu of the plasma, which has a continuous influence upon the physiology of the cells, we can assume that the plasma must have played a role in the emergence of the cells with this potential. The exact plasma constituent remains to be determined; however, some component of diabetic plasma is a likely candidate.

An interesting but puzzling finding was that monocytes from the diabetic-hypertriglyceridemic, hypertriglyceridemic, and healthy groups were able to transform better in the heterologous normal serum system than in the autologous serum system. This observation points to the possibility that there may be some undefined mechanism that suppresses spontaneous differentiation of monocytes into macrophages and is specific to individuals. If this mechanism relates to some aspect(s) of the immune system, then the mechanism of monocyte differentiation as well as the role that the monocyte-derived macrophages play in atherogenesis may require investigations involving cellular immunology.

In the present study, only lipid metabolism in monocyte-derived macrophages was investigated. However, the results of the investigation raise the possibility that other cells, if not all, that are thought to be involved in atherogenesis may also be modified or selected by being exposed to plasma with a particular composition, such as diabetic, hypertriglyceridemic, or hypercholesterolemic plasma. To what extent these cells deviate from cells that are bathed in plasma of normal composition should be examined.

We thank Miss Y. Sei for her technical assistance and Prof. Wilfred Y. Fujimoto for reviewing the manuscript. This study was supported in part by a grant-in-aid for research (C-61570562) from The Japanese Ministry of Education, Science, and Culture of Japan and grants

from The Japan Heart Foundation and the Arima Memorial Foundation for Medical Research.

REFERENCES

1. Garcia, M.J., MacNamara, P.M., Gordon, T., and Kannell, W.B. (1974): Morbidity in diabetics in the Framingham population: Sixteen-year follow-up study. *Diabetes*, **23**, 105-111.
2. Santen, R.J., Willis, P.W., and Fajans, S.S. (1972): Atherosclerosis in diabetes mellitus: Correlations with serum lipid levels, adiposity and serum insulin levels. *Arch. Intern. Med.*, **130**, 833-843.
3. Lopes-Virella, M.F., Klein, R.L., Lyons, T.J., and Stevenson, H.C. (1988): Glycosylation of low-density lipoprotein enhances cholesteryl ester synthesis in human monocyte-derived macrophages. *Diabetes*, **37**, 550-557.
4. Guanterco, S.H., Bradley, W.A., and Gotto, A.N. (1982): Hypertriglyceridemic very low density lipoproteins induce triglyceride synthesis. *J. Clin. Invest.*, **70**, 168-178.
5. Hiramatsu, K., and Arimori, S. (1988): Increased superoxide production by mononuclear cells of patients with hypertriglyceridemia and diabetes. *Diabetes*, **37**, 832-837.
6. Aqel, N.M., Ball, R.Y., Waldman, H., and Mitchinson, M.J. (1984): Monocytic origin of foam cells in human atherosclerotic plaques. *Atherosclerosis*, **53**, 265-271.
7. National Diabetes Data Group (1979): Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. *Diabetes*, **28**, 1039-1057.
8. Lindergren, F.T., and Jensen, L.C. (1972): The isolation and quantitative analysis of serum lipoproteins, in *Blood Lipids and Lipoproteins: Quantitation, Composition and Metabolism*, ed. by Nelson, G.J., Wiley-Interscience Inc., New York, pp. 181-274.
9. Basu, S.K., Goldstein, J.L., Anderson, R.G.W., and Brown, M.S. (1976): Degradation of cationized low density lipoprotein and regulation of cholesterol metabolism in homozygous familial hypercholesterolemia fibroblasts. *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 3178-3182.
10. Langer, T., Strober, W., and Levy, R.L. (1972): The metabolism of low density lipoprotein in familial type II hyperlipoproteinemia. *J. Clin. Invest.*, **51**, 1528-1536.
11. Folch, J., Lees, M., and Stanley, G.H.S. (1957): A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, **226**, 497-509.
12. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, B.J. (1951): Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, **193**, 265-275.
13. Hiramatsu, K., Bierman, E.L., and Chait, A. (1985): Metabolism of low-density lipoprotein from patients with diabetic hypertriglyceridemia by cultured human skin fibroblasts. *Diabetes*, **34**, 8-14.
14. Snedecor, G.W., and Cochran, W.G. (1967): *Statistical Methods*, Iowa State University Press, Iowa City, pp. 93-99.
15. Vlassara, H., Brownlee, M., and Cerami, A. (1988): Specific macrophage receptor activity for advanced glycosylation end products inversely correlates with insulin levels in vivo. *Diabetes*, **37**, 456-461.
16. Angelin, B. (1988): Regulation of 3-hydroxy-3-methylglutaryl-coenzyme A reductase activity in mouse peritoneal macrophages. *Biochem. J.*, **255**, 529-534.
17. Kraemer, F.B. (1986): Insulin deficiency alters cellular cholesterol metabolism in murine macrophages. *Diabetes*, **35**, 764-770.