Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 9010–9014, November 1990
Medical Sciences

Advanced protein glycosylation induces transendothelial human monocyte chemotaxis and secretion of platelet-derived growth factor: Role in vascular disease of diabetes and aging

(arteriosclerosis/atherosclerosis/macrophages/migration/tissue remodeling)

M. KIRSTEIN*, J. BRETT†, S. RADOFF*, S. OGAWA†, D. STERN†, AND H. VLASSARA*

*Laboratory of Medical Biochemistry, The Rockefeller University, New York, NY 10021; and †Department of Physiology, Rover Physiology Laboratories, Columbia University, College of Physicians and Surgeons, New York, NY 10032

Communicated by Maclyn McCarty, August 20, 1990

ABSTRACT **Diabetes and aging are commonly accompanied by arterio- and atherosclerosis. Infiltration of the arterial subendothelial intima by macrophages/monocytes is an important early event preceding the development of atheromatous lesions; these macrophages are known to produce mitogenic factors in early atherosclerotic lesions. It has been previously shown that, over time, vascular matrix accumulates proteins nonenzymatically modified by advanced glycosylation end products (AGEs). In view of the fact that macrophages/monocytes have AGE-specific receptors associated with the expression of several growth factors, we investigated the possibility that AGEs mediate initial monocyte–vessel wall interactions that occur before overt formation of vascular lesions. This study demonstrates that (*i*) *in vitro*- and *in vivo*-formed AGEs are chemotactic for human blood monocytes, (*ii*) subendothelial AGEs can selectively induce monocyte migration across an intact endothelial cell monolayer, and (*iii*) subsequent monocyte interaction with AGE-containing matrix results in the expression of platelet-derived growth factor. These results support the existing hypothesis that *in vivo*-forming glucose-derived protein adducts can act as signals for the normal turnover of senescent tissue protein by means of the AGE-specific receptor system. Time-dependent glucose-induced deposition of AGEs on matrix proteins may promote monocyte infiltration into the subendothelium. Subsequent AGE-triggered macrophage activation and consequent elaboration of proliferative factors may normally coordinate remodeling but may also lead to the diverse pathogenic changes typical of arterio- and atherosclerosis in diabetic or aging populations.**

Proteins exposed to ambient glucose undergo nonenzymatic glycosylation, leading to the progressive formation of highly reactive late addition products termed advanced glycosylation end products (AGEs). Steady accumulation of AGEs on proteins with relatively long half-lives in many tissues, especially on subendothelial basement-membrane proteins, occurs with normal aging (1) and at an accelerated rate in diabetes (2), suggesting a possible etiologic role in the pathogenesis of the vascular complications associated with these conditions.

Although monocytes are involved in the normal maintenance and remodeling of the vascular basement membrane (3), they have also been causally implicated in the pathogenesis of vascular lesions in atherosclerosis (4–6). Monocytes/macrophages express AGE-specific receptors that mediate endocytosis and degradation of AGE-modified proteins (7). After AGE binding and intracellular processing, monocytes synthesize and secrete growth-promoting cytokines such as cachectin/tumor necrosis factor, interleukin 1, and insulin-like growth factor 1 (8, 9). Because this mechanism could be fundamental to vessel-wall tissue homeostasis before the manifestations of vascular pathology, it was important to determine whether AGEs promoted monocyte–vessel wall interactions, leading to monocyte migration into the subendothelium and the subsequent activation of these monocytes.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Preparation of AGE-Modified Bovine Serum Albumin. AGE–bovine serum albumin (BSA) was prepared by incubating BSA (fraction V, low endotoxin; Boehringer Mannheim) in phosphate-buffered saline (PBS) with 50 mM glucose 6-phosphate (Glc-6-*P*) as described (7). Normal control BSA was incubated under the same conditions without Glc-6-*P*. Fluorescence spectra of AGE–BSA (at 460 nm upon excitation at 390 nm) indicated increased glycosylation as a function of time (see *Results*). At 42 days AGE–BSA contained 80 arbitrary fluorescence units (AFU per 100 μg of protein), whereas unmodified BSA maintained background levels (4 AFU per 100 μg of protein). For selected experiments samples (20 mg each) incubated with 50 mM Glc-6-*P* for 3 and 6 days were reduced with sodium borohydride as described (10).

Preparation of AGE-Modified Low Density Lipoprotein (LDL). LDL was prepared from pooled normolipidemic human serum by density-gradient ultracentrifugation (11). LDL was glycosylated *in vitro* (AGE–LDL) as described above for 3 weeks in the presence of the antioxidants butylated hydroxytoluene (20 μM) and EDTA (0.5 mM) and under a blanket of N_2 in sealed tubes. Oxidized LDL was prepared by incubating LDL for 3 weeks in the absence of antioxidants or glucose. The amount of lipid peroxidation was estimated as thiobarbituric acid-reactive material (12), and the extent of advanced nonenzymatic glycosylation of the LDL samples was determined by fluorescence as described above. AGE-modified LDL exhibited three times greater fluorescence (30 AFU per 100 μg of protein) than unmodified LDL (9 AFU per 100 μg of protein).

Preparation of Peripheral Nerve Myelin. Human peripheral nerve myelin obtained at autopsy from a 5-month-old normal infant, a 63-year-old nondiabetic, and a 61-year-old diabetic was isolated by standard procedures (13). Fluorescence studies of the myelin from the 61-year-old diabetic indicated 3-fold greater AGE-levels (156 AFU per 100 μg of protein) than the age-matched normal individual (50 AFU per 100 μg of protein), which produced 3.5-fold greater fluorescence

Abbreviations: AGEs, advanced glycosylation end products; FMLP, *N*-formylmethionylleucylphenylalanine; LDL, low density lipoprotein; PDGF, platelet-derived growth factor; BSA, bovine serum albumin; AFU, arbitrary fluorescence units; GBSS, Gey's balanced salt solution; Glc-6-*P*, glucose 6-phosphate.

intensity than the infant's background level (14 AFU per 100 μg of protein).

Chemotaxis Assays. Human peripheral blood monocytes were isolated from healthy volunteers using Ficoll/Hypaque and Percoll gradients (14, 15) and were suspended in Gey's balanced salt solution (GBSS)/20 mM Hepes/2% BSA at 2.5 × 10^6 cells per ml. Glycosylated proteins were diluted in GBSS, and chemotaxis was assayed in a 48-well modified Boyden micro-chemotaxis chamber (Neuro Probe, Cabin John, MD), as described (16).

N-formylmethionylleucylphenylalanine (FMLP; Peninsula Laboratories), a synthetic peptide highly chemotactic for monocytes, served as a positive control; GBSS served as the negative control (16). Chemotactic activity was defined as the mean number (± SEM) of monocytes that had migrated in response to the test substance, averaged across four high-power fields (×500) for each of triplicate chemotaxis chamber filters minus the number of cells that had migrated in the presence of negative-control GBSS alone. The differences among means of all the modified protein samples and the unmodified samples were all statistically significant at $P < 0.002$ with Student's *t* test.

Assessment of Monocyte–Endothelial Cell Interactions. Monolayers of human umbilical vein endothelial cells were grown to confluence on a substrate composed of type IV murine collagen (Collaborative Research) and native BSA (Sigma) or AGE–albumin, either of which was mixed at equal protein concentration with the collagen. Monocytes, isolated as described above, were vitally stained with rhodamine phalloidin (pH 26), a lipophilic membrane dye (Xynaxis, Malvern, PA) and then added to the endothelial cultures (2.5–3.0 × 10^5 cells per cm^2) for 3 hr at 37°C. Monolayers were then washed and fixed with 2% paraformaldehyde/PBS, pH 7.2, for immunofluorescence. The confluent endothelial monolayer was visualized by staining with fluorescence isothiocyanate-conjugated *Ulex europaeus* lectin (17).

Electron Microscopy Studies. Confluent endothelial monolayers were grown on a matrix composed of AGE–albumin conjugated to colloidal gold (25 nm) (18) and type IV collagen and exposed to monocytes as described above. At the end of incubation, monolayers were fixed for electron microscopy as described (19).

Monocyte-Associated Platelet-Derived Growth Factor (PDGF). Monocytes, incubated overnight on matrix composed of collagen IV mixed with native albumin or AGE–albumin, were visualized by rhodamine phalloidin staining. Monocyte-associated PDGF was detected by indirect immunofluorescence with the use of a goat polyclonal anti-human PDGF (A and B chain) antibody (Collaborative Research) visualized with fluorescein isothiocyanate-labeled rabbit anti-goat immunoglobulin (Sigma). Immunoblotting was done as described (20) to assess PDGF expression by human monocytes that were plated on albumin-containing or AGE–albumin-containing matrix (prepared as described above) and incubated for 18 hr under serum-free medium. Conditioned media (in each case from ≈10^6 cells) were immunoprecipitated with a monospecific rabbit anti-human PDGF antiserum (Collaborative Research) as described (20). PDGF antigen was visualized by reacting the blot sequentially with rabbit anti-human PDGF antiserum and radioiodinated affinity-purified anti-rabbit immunoglobulin (Sigma).

RESULTS

***In Vitro*-Glycosylated BSA (AGE–BSA) Is Chemotactic for Human Monocytes.** Monocyte migration to a model ligand, AGE–albumin, was measured in modified Boyden chambers. Monocyte migration as a function of AGE–BSA concentration is shown in Fig. 1*A*. Directed migratory activity was detectable at a protein concentration of 0.5 μg/ml, whereas

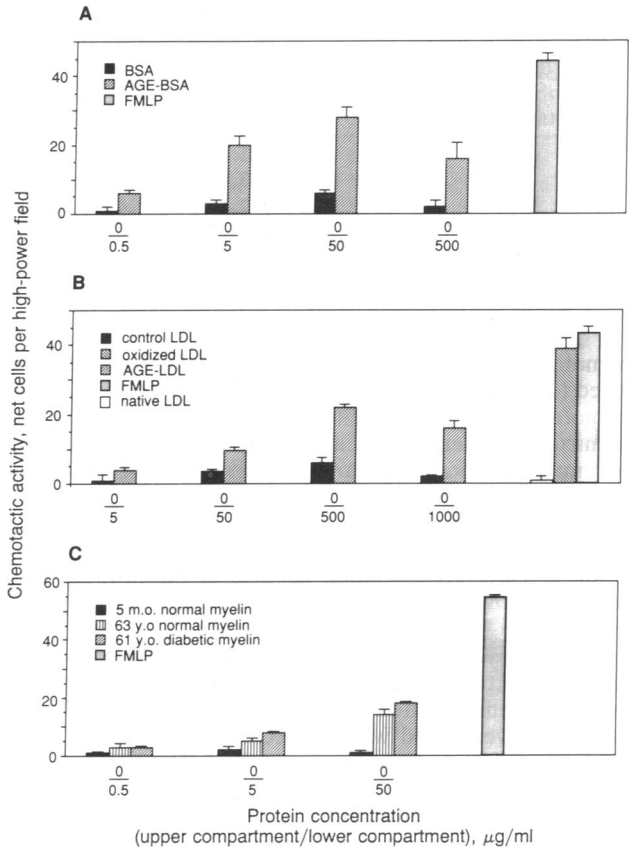

FIG. 1. Monocyte chemotaxis to AGE–proteins. Monocyte migration was measured in response to increased concentrations of unmodified BSA and *in vitro* glucose-modified BSA (AGE–BSA) (*A*), unmodified control LDL and *in vitro* glucose-modified LDL (AGE–LDL) (*B*); oxidized LDL (50 μg/ml) served as a positive control, and freshly isolated native LDL (50 μg/ml) was the negative control. (*C*) *In vivo*-glycosylated myelin from 63-year-old normal, a 61-year-old diabetic, and a 5 month-old normal subject. FMLP at 10 ng/ml served as positive control.

maximal cell migration occurred at an AGE–albumin concentration of 50 μg/ml, reaching ≈60% of the maximal response to a known monocyte chemotaxis control, FMLP. At higher concentrations of AGE–BSA, directed migration declined, a phenomenon described with other chemotactic substances (21). Unmodified BSA did not enhance any migratory activity by human monocytes. Chemotactic activity of AGE–albumin preparations was retained after extensive digestion by proteinase K (100 μg/mg of albumin), indicating that higher order protein structural features were not involved, as shown for albumin after maleylation (22) or single lysine glycosylation (23). Checkerboard analysis confirmed that movement of monocytes in the presence of AGE–albumin represented directed chemotaxis and not enhanced random cell migration (chemokinesis) (Table 1): monocyte migration was enhanced only when the concentration of AGE–albumin in the lower compartment exceeded that in the upper compartment. The observation that AGE–albumin enhanced neither chemotaxis nor chemokinetic activity of peripheral blood polymorphonuclear leukocytes (data not shown) confirmed the specificity of this activity for mononuclear cells.

At constant temperature, pH, and glucose concentration AGE formation on proteins increases with time (2). To correlate the formation of AGEs on BSA with its chemotactic activity, AGE accumulation measured by specific fluorescence was plotted against chemotactic response (Fig. 2). A nearly linear relationship between AGE formation and

Table 1. Checkerboard analysis of monocyte chemotactic response to AGE–BSA

AGE–BSA in lower compartment, μg/ml	AGE–BSA in upper compartment			
	0 μg/ml	5 μg/ml	50 μg/ml	500 μg/ml
5	20 ± 1.8	1 ± 1.1	−1 ± 1.1	2 ± 1.1
50	28 ± 1.8	15 ± 3.4	−1 ± 1.1	2 ± 1.1
500	16 ± 3.3	9 ± 2.1	4 ± 1.4	2 ± 2.0

Data are presented as net (minus GBSS/GBSS control wells = 13 ± 1.1) cells per high-power field ± SEM (at ×500 magnification). FMLP (10 nM) served as the positive control with a net migration of 49 ± 0.7 cells per high-power field.

chemotaxis was observed. Although AGE fluorescence continued to accumulate with longer incubation times, maximal chemotactic activity was achieved by 16 days, indicating that submaximal protein modification by glucose was sufficient to elicit maximal migratory activity in this system. To study the chemoattractant potential of early glycosylation products, AGE-albumin was reduced at various time points with sodium borohydride (10), thus eliminating the possible contribution of early glycosylation adducts of the protein. Such treatment, however, did not affect chemotactic activity (data not shown).

***In Vitro*-Glycosylated LDL Is Chemotactic for Monocytes.** Extensive extracellular accumulation of extravasated LDL has been demonstrated in the arterial subintima (2). We therefore investigated whether AGE modification of LDL, under conditions selected to minimize LDL oxidation, rendered the lipoprotein effective in attracting monocytes. Fig. 1*B* shows that AGE-modified LDL prepared in the presence of antioxidants (12) at a concentration of 500 μg/ml was approximately three times more chemotactic for monocytes than nonglycosylated LDL handled with similar precautions, whereas freshly isolated native LDL showed no such activity. LDL incubated in the absence of oxidation inhibitors was almost as chemotactic as the highly chemotactic FMLP. Many investigators have implicated oxidized lipoprotein as initiating monocyte recruitment into the vessel wall (24, 25).

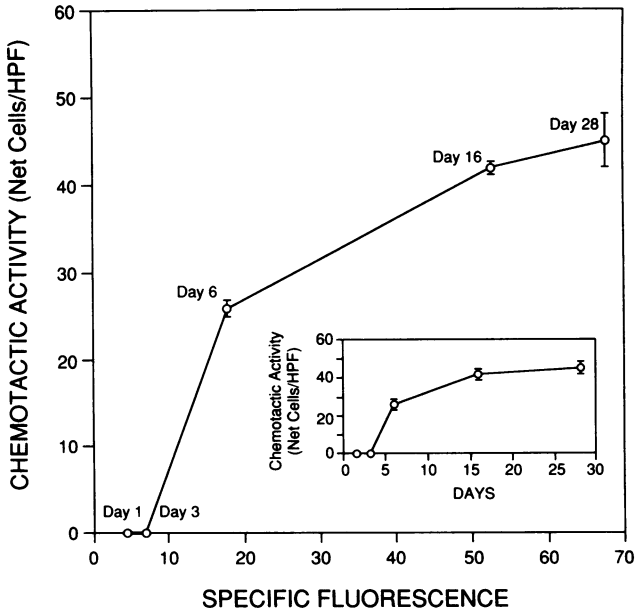

FIG. 2. Relationship between chemotactic activity and *in vitro* AGE formation (measured as relative fluorescence) as a function of time. BSA was incubated with Glc-6-*P* for up to 28 days, and specific fluorescence was assessed. Chemotactic activity of the AGE–BSA samples at a concentration of 50 μg/ml was measured. (*Inset*) Chemotactic activity of AGE–BSA as function of exposure time to Glc-6-*P*. HPF, high-power field.

Table 2. Inhibition of antioxidants of phospholipid hydrolysis during LDL incubation

LDL incubation conditions	Incubation period	TBA-reactive material, nmol of malondialdehyde per 100 μg of protein
Fresh, no inhibitors	0 days	0.412
No inhibitors	3 weeks	4.420
20 μM BHT + 500 μM EDTA	3 weeks	0.448
50 mM Glc-6-*P* + 20 μM BHT + 500 μM EDTA	3 weeks	0.448

BHT, butylated hydroxytoluene; TBA, thiobarbituric acid.

To be certain that oxidized LDL present in the AGE–LDL preparations was not responsible for monocyte migration under these conditions, the oxidation state of all samples was assessed (12) (Table 2). Only LDL incubated in the absence of antioxidants showed significant lipid peroxidation [the latter material was chemotactic for monocytes as reported (24)], whereas in their presence lipid peroxidation was almost completely prevented (Table 2).

***In Vivo*-Glycosylated Myelin Proteins Are Chemotactic for Monocytes.** The capacity of *in vivo*-formed AGEs to induce monocyte migration was tested by using myelin proteins isolated from human peripheral nerves. Monocyte attraction toward myelin from a 61-year-old diabetic was compared with myelin from a nondiabetic individual of the same age and

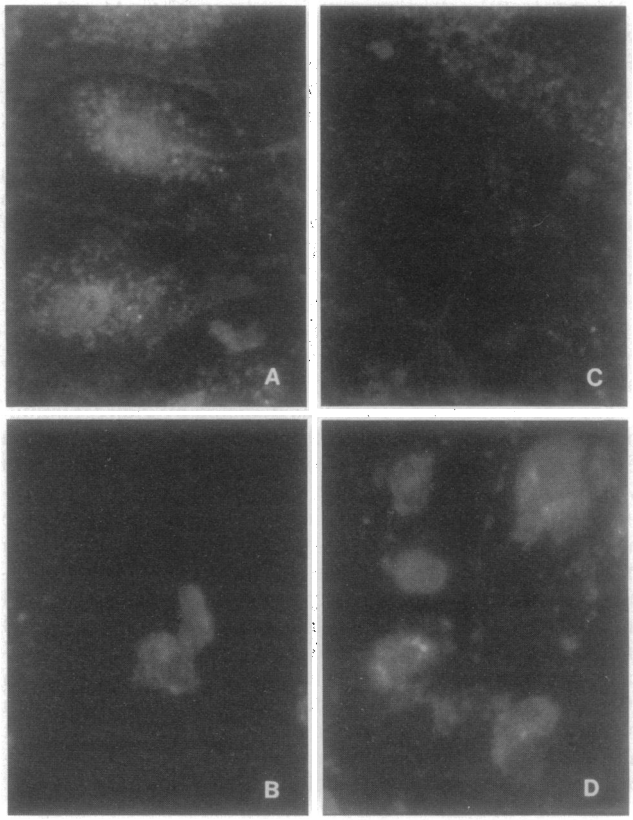

FIG. 3. Effect of AGEs on endothelial cell–monocyte interaction. Monolayers of human umbilical vein endothelial cells were grown to confluence on a substrate composed of native albumin (*A* and *B*) or AGE–albumin (*C* and *D*). (*A* and *C*) The confluent endothelial monolayer was visualized by staining with fluorescein isothiocyanate-conjugated *Ulex europaeus* lectin. (*B* and *D*) Same fields as *A* and *C*, respectively. Rhodamine phalloidin-stained monocytes are evident below the monolayer in the subendothelial matrix. (×480.)

from a 5-month-old normal infant (Fig. 1*C*). Although the infant-derived myelin was chemotactically inactive, the diabetic and age-matched normal myelin were each chemoattractive for monocytes at 50 μg/ml.

Effect of AGEs on Endothelial Cell–Monocyte Interaction. To be implicated either in normal tissue turnover or in the pathogenesis of early preatherosclerotic lesions, AGEs in subendothelial matrix would have to selectively induce migration of monocytes across the endothelial monolayer. A model system of these interactions, comprising freshly isolated blood monocytes with human umbilical vein endothelial cell monolayers previously grown to confluence over matrices mixed with either native albumin or AGE–albumin, is shown in Fig. 3. Endothelial cells seeded onto matrix of collagen type IV prepared with unmodified native albumin formed a monolayer made visible by the fluorochromed lectin of *Ulex europeaus* (17) (Fig. 3*A*). Few of the monocytes introduced on top of these monolayers migrated across the endothelium (Fig. 3*B*). In contrast, monocytes seeded onto endothelial monolayers grown on matrices of collagen type IV prepared with AGE–albumin (Fig. 3*C*) penetrated through the monolayer >3-fold that on native albumin (Fig. 3*D*). In other experiments, transendothelial monocyte migration was observed when AGE–albumin was preincubated with the endothelial monolayer for 24 hr, a period sufficient to allow for endothelial transcytosis and deposition of AGEs in the matrix (data not shown).

Visualization of Monocytes Migrating Through the Endothelial Monolayer. Monocytes were visualized migrating through the endothelial monolayer at sites where AGE–gold was concentrated (Fig. 4). Once the monocytes had crossed between endothelial cells into the matrix, continuity of the endothelial monolayer was restored (Fig. 4*B*). In contrast to AGE-induced migration of monocytes across the endothelial monolayer, polymorphonuclear leukocytes were not attracted across the monolayer at an enhanced rate in the presence of AGEs, demonstrating that the chemotactic signal was selective for monocytes (data not shown).

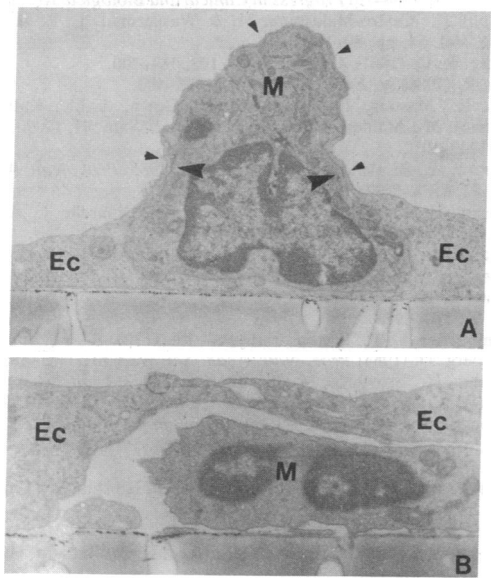

FIG. 4. Migration of monocytes across endothelial monolayers and their relationship to subendothelial AGE deposits. (*A*) Monocyte transit across endothelial monolayer; the monocyte has been completely enveloped apically by cytoplasmic extensions (small black arrowheads) of the endothelial cell(s) between which it is migrating and appears as nested in the endothelium, with membranes of both cells closely apposed (large arrowheads). Deposits of AGE–albumin–gold conjugates are evident in the subendothelial matrix. (*B*) Monocyte beneath the endothelial monolayer is flattened and apparently migrating toward an AGE-albumin-gold deposit. M, monocyte; Ec, endothelial cell. The circle at surface of Ec in *B* is an artifact. (×5360.)

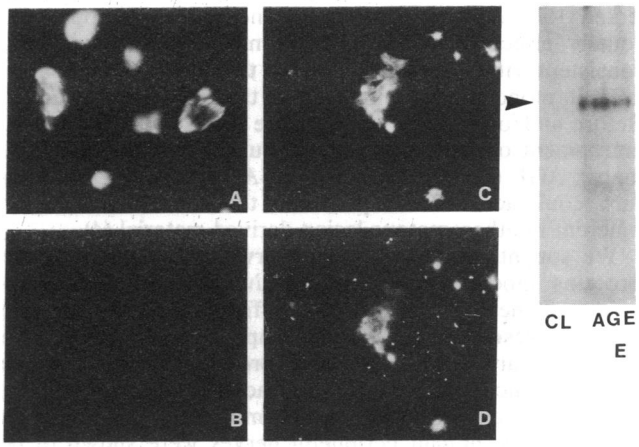

FIG. 5. PDGF in monocytes on matrices containing AGEs. Peripheral blood monocytes, incubated overnight on a model matrix composed of collagen mixed with native albumin (*A* and *B*) or AGE–albumin (*C* and *D*) were visualized by rhodamine phalloidin staining (*A* and *C*), and monocyte-associated PDGF (*B* and *D*) was detected by indirect immunofluorescence. (*E*) Immunoblotting demonstrated PDGF secretion (31- to 35-kDa band indicated by arrowhead) by monocytes after incubation in an AGE–albumin-containing model matrix (AGE) but not on an unmodified model matrix (CL). (×384.)

AGE-Modified Matrix Proteins Induce PDGF Expression in Monocytes. It has previously been shown that AGEs induce monocytes/macrophages to produce cytokines, such as cachectin/tumor necrosis factor, and interleukin 1 (8), as well as insulin-like growth factor 1 (9). PDGF, a mitogen for vascular smooth muscle cells, is believed to play a central role in the development of proliferative atherosclerotic lesions (5, 6). In this context, monocytes exposed to a culture surface coated with native albumin/collagen type IV, spread, but did not express detectable PDGF (Fig. 5 *A* and *B*). In contrast, on a surface coated with AGE–albumin/collagen type IV, monocytes stained positively for PDGF by immunofluorescence using a goat polyclonal anti-human PDGF (A and B chain) antibody (Fig. 5 *C* and *D*). Consistent with these results, immunoblotting demonstrated PDGF expression only in monocytes exposed to AGE-containing surface versus the normal control (Fig. 5*E*). At this point we cannot distinguish which form of PDGF was present in the conditioned medium from AGE-adherent monocytes/macrophages because our studies employed a polyclonal antibody to PDGF.

DISCUSSION

Macrophages/monocytes are thought to play a significant role in the host response to the deposition of AGEs in tissues (8). In turn, the ever present AGEs, through AGE-specific receptors, may regulate tissue turnover and repair (8, 9). In conditions such as diabetes and aging, where increased AGE formation prevails, the balance between AGE formation and tissue turnover may be disturbed. However, one step before macrophage influx into the tissues, the chemotactic responses and the interactions of AGEs with endothelial cells are of primary importance. In this report AGEs are shown to be very effective, as well as selective, mediators of monocyte migration. *In vitro* glucose-derived modification of albumin produced a dose-dependent chemotactic response of monocytes, unlike its unmodified counterpart. AGE modification of an unrelated macromolecule, LDL, under conditions se-

lected to minimize oxidation, rendered the lipoprotein equally effective in attracting monocytes. This result is consistent with the widely held view that implicates modified LDL in monocyte recruitment into the vessel wall (24, 25). Before and/or parallel with oxidase modification, covalent entrapment of the largely extracellular lipoprotein through matrix AGE (2) would allow AGE–Apo B to form. This, in turn, could account for a portion of the chemotactic activity inherent in atheromatous lesion-derived material (4).

We sought to confirm our observations by using tissue proteins modified by AGE *in vivo*. Vessel-wall AGE-mediated chemotactic activity is difficult to dissect from the activity present in the native components. Therefore, we turned toward a different tissue protein, peripheral nerve myelin, which, unlike collagen, lacks significant inherent chemotactic activity. *In vivo* AGE-modified myelin proteins isolated from human peripheral nerves were shown to be chemotactic for monocytes. Myelin from either a 61-year-old diabetic or an age-matched normal individual was equally chemotactic for monocytes as compared with chemotactically inactive myelin from a normal infant. This result was consistent with the fluorescence analysis data, indicating that compared with minimal AGE levels in the infant's myelin sample, both the 61-year-old diabetic and the age-matched normal myelin contained far greater AGE levels (11- and 3.5-fold, respectively) as reported (26). Although the AGE content of the diabetic myelin was greater than that from the normal individual of the same age, the corresponding monocyte chemotactic activity was similar, consistent with the observation that glucose modification beyond a certain extent does not further enhance migratory activity. These observations lend support to the notion that once proteins are exposed to ambient glucose *in vivo* beyond a certain time period, they acquire AGEs sufficient to signal monocyte/macrophage mobilization for their removal and replacement. This may explain, in part, the ubiquitous presence of these cells in diverse tissues, including vessel wall and peripheral nerves (5–7, 26).

AGEs could accumulate in the vascular subendothelial basement membrane by two mechanisms: directly, by the time- and glucose-dependent formation of AGEs on long-lived basement-membrane components such as collagen (1) and indirectly, via the endothelial-cell receptor-mediated uptake, transcytosis, and deposition of plasma AGEs into basement-membrane matrix (19). However, to be implicated either in tissue turnover or in the pathogenesis of early preatherosclerotic lesions, AGEs in subendothelial matrix would have to selectively induce migration of monocytes across the endothelial monolayer. Indeed, as the cells forming the luminal vascular surface, endothelial cells constitute a dynamic barrier that regulates permeability, hemostasis, and interactions of the vessel wall with circulating cells. AGEs have been demonstrated to lead to changes in permeability and cell-surface coagulant properties of endothelial cells (19). Here we show that monocytes, seeded onto endothelial monolayers grown on AGE-containing matrices, penetrated through the monolayer in greater numbers, whereas few monocytes were attracted to unmodified native matrices. Monocytes could also be visualized migrating through the endothelial monolayer at sites where AGE–gold was concentrated, after which continuity of the monolayer was restored. This is consistent with the observations made during early stages of atherogensis, when the endothelium appears intact, although monocyte infiltration has already occurred (5).

Monocytes in the subendothelial space, when activated, can produce a spectrum of mediators capable of attracting and of stimulating other cell types, thus eventually changing drastically the architecture of the vessel wall (5, 6). Here we show that monocytes seeded onto a surface coated with AGE-containing matrix express PDGF. This is an important finding because PDGF expressed in vessel-wall macrophages in all stages of lesion development (6) is mitogenic for vascular smooth muscle cells and is believed to play a central role in the development of proliferative atherosclerotic lesions (5, 6). Thus, ubiquitously present AGEs may act as a signal for tissue turnover and cell proliferation in advance of the development of frank atheromatous lesions.

The mechanism proposed in the current communication introduces the possibility that spontaneous glucose-mediated vessel-wall protein modification may initiate monocyte migration and activation long before endothelial-cell injury or lipid abnormalities are manifested. In normal tissues with minimal AGE levels, low degrees of monocyte migration and activation might not result in any pathological consequences but rather serve as part of an efficient protein-turnover apparatus. With advanced age (8) or in the presence of hyperglycemia (2), the enhanced expression of monokines with proliferative effects, induced by the interaction of activated macrophages with high levels of subendothelial AGE and oxidized LDL, could set the stage for subsequent pathologic events, such as induction of smooth muscle-cell migration and proliferation (5).

Human peripheral nerve tissue was provided by the National Disease Research Interchange (Philadelphia). This work was supported, in part, by National Institutes of Health Grants AGO 8245 and AGO 6943 and by grants from the U.S. Public Health Service (HL34625, HL42833, and HL42507), the Council for Tobacco Research (1971 and 2101R1), and the New York Lung Association. D.S. contributed to this work during the tenure of a Genentech–American Heart Association Established Investigator Award. M.K. is a fellow of the New York State Health Research Council.

1. Kohn, R. R., Cerami, A. & Monnier, V. M. (1984) *Diabetes* **33**, 57–59.
2. Brownlee, M., Cerami, A. & Vlassara, H. (1988) *N. Engl. J. Med.* **318**, 1315–1321.
3. Krane, S. M. (1984) in *Progress in Clinical and Biological Research*, eds. Berk, P. D., Castro-Malaspina, H. & Wasserman, L. R. (Liss, New York), Vol. 54, pp. 89–102.
4. Gerrity, R. G. (1981) *Am. J. Pathol.* **103**, 181–200.
5. Ross, R. (1986) *N. Engl. J. Med.* **314**, 488–500.
6. Ross, R., Masuda, J., Raines, E. W., Gown, A. M., Katsudo, S., Sasahara, M., Malden, L. T., Masuko, H. & Sato, H. (1990). *Science* **248**, 1009–1012.
7. Vlassara, H., Brownlee, M. & Cerami, A. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 5588–5592.
8. Vlassara, H., Brownlee, M., Manogue, K. R., Dinarello, C. A. & Pasagian, A. (1988) *Science* **240**, 1546–1548.
9. Kirstein, M., Aston, C. & Vlassara, H. (1990) *FASEB J.* **4**, A1759 (abstr.).
10. Koenig, R. J. & Cerami, A. (1980) *Annu. Rev. Med.* **31**, 29–34.
11. Chung, B. H., Wilkinson, T., Geer, J. C. & Segres, J. P. (1980) *J. Lipid Res.* **21**, 284–291.
12. Steinbrecher, U. P., Parthasarathy, S., Leake, D. S., Witztum, J. L. & Steinberg, D. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 3883–3887.
13. Vlassara, H., Brownlee, M. & Cerami, A. (1983) *Diabetes* **32**, 670–674.
14. Boyum, A. (1968) *Scand J. Clin. Lab. Invest.* **21**, Suppl. 97, 77–89.
15. Timonen, T. & Saksela, E. (1980) *J. Immunol. Methods.* **36**, 285–291.
16. Falk, W., Goodwin, R. H. Jr., & Leonard, E. J. (1980) *J. Immunol. Methods* **33**, 239–247.
17. Holthöfer, H., Virtanen, I., Kariniemi, A. L., Hormia, M., Linder, E. & Miettinen, A. (1982) *Lab. Invest.* **47**, 60–66.
18. Handley, D. & Chien, S. (1983) *Proc. Soc. Exp. Biol. Med.* **174**, 1–11.
19. Esposito, C., Gerlach, H., Brett, J., Stern, D. & Vlassara, H. (1989) *J. Exp. Med.* **170**, 1387–1407.
20. Stern, D., Brett, J., Harris, K. & Nawroth, P. (1986) *J. Cell Biol.* **102**, 1971–1978.
21. Schiffmann, E., Corcoran, B. & Wahl, S. M. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 1059–1062.
22. Haberland, M. E., Rasmussen, R. R. & Fogelman, A. M. (1986) *J. Clin. Invest.* **78**, 827–831.
23. Shaklai, N., Garlick, R. L. & Bunn, H. F. (1984) *J. Biol. Chem.* **259**, 3812–3817.
24. Quinn, M. T., Parthasarathy, S., Fong, L. G. & Steinberg, D. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 2994–2998.
25. Steinberg, D., Parthasarathy, S., Carew, T. E., Khoo, J. C. & Witztum, J. L. (1989) *N. Engl. J. Med.* **320**, 915–923.
26. Vlassara, H., Brownlee, M. & Cerami, A. (1985) *Diabetes* **34**, 553–557.