Placenta (1997), 18, 249–253

Immunohistochemical Localization of Lipoperoxidation Products in Normal Human Placenta

A. Casasco[a,c], A. Calligaro[a], M. Casasco[a], S. Tateo[b], A. Icaro Cornaglia[a], M. Reguzzoni[a] and A. Farina[a]

[a] Institute of Histology and Embryology, University of Pavia, via Forlanini 10, 1-27100 Pavia, Italy

[b] Obstetrics and Gynecology Clinic, I.R.C.C.S. 'San Matteo' Hospital, 1-27100 Pavia, Italy

Paper accepted 31 October 1996

4-Hydroxynonenal (4-HNE) is a major propagation product of lipid peroxidation that is supposed to be responsible for some of the effects associated with oxidative stress in tissues. We have investigated the possible occurrence and distribution of 4-HNE-immunoreactivity in human normal placenta using immunocytochemistry. Specific immunostaining was observed in cytotrophoblast cells, syncytiotrophoblast, some cells of the villous mesenchyme and some endothelial cells of first trimester and term placentae. The detection of 4-HNE-immunoreactivity in placenta raises the question whether lipoperoxidation products are produced locally in placental cells or represent exogenous products that derive from maternal blood flow. Since trophoblastic cells and villous macrophages are provided by a scavenger receptor, it is conceivable that these cells may play a protective role with regard to the diffusion of lipoperoxidation products from the mother to the embryo. However, since a significant degree of lipid oxidative modification does not take place in plasma, it is presumed that 4-HNE is a local product of placental metabolism. In line with this hypothesis, it is proposed that maternal low density lipoproteins, which are the major source of cholesterol for placental steroid synthesis, might be oxidized by villous cells during their traversal through the villous wall.

Placenta (1997), 18, 249–253 © 1997 W. B. Saunders Company Ltd

INTRODUCTION

Human placental cells interact with all of the major classes of normal circulating lipoproteins, and lipoprotein metabolism is related to fetal growth and development (Knopp et al., 1985, 1991). Recently, scavenger receptor activity has been demonstrated in placental cells in vitro, thus suggesting that placenta may bind and metabolize also modified lipoproteins (Malassiné et al., 1990; Bonet et al., 1995). There is also evidence that lipoprotein oxidation may be enhanced in pregnancy (Ishihara, 1978; Hubel et al., 1989) and it has been presumed reasonably that normal lipoproteins might be oxidized by trophoblast cells (Bonet et al., 1995).

Polyunsaturated fatty acids (PUFA), major components of all circulating lipoproteins, are amongst the most susceptible molecules to oxidative stimuli within the cell. During the oxidative degradation of PUFA, a variety of reactive aldehydic products—including 4-hydroxynonenal (4-HNE) and malondialdehyde—are formed. These products are capable of attaching covalently to protein, particularly to the epsilon amino groups of lysine residues, thus producing important biological actions (for a review see Estebauer, Schaur and Zollner, 1991). Because of its stability, 4-HNE, as a major propagation product of lipid peroxidation, represents a suitable epitope to reveal the sites of lipid peroxidation using immunochemical methods (Yla-Herttuala et al., 1989; Palinski et al., 1990; Rosenfeld et al., 1990).

The aim of our study was to investigate the possible occurrence and distribution of 4-HNE-immunoreactivity in human normal placenta using immunohistochemistry.

MATERIALS AND METHODS

Sample collection and preparation

First trimester human placentae (n=13) were collected from legal abortions at 8–12 weeks of pregnancy. Reasons for interruptions were unlikely to affect placental function and structure. Full-term placentae (n=23) were obtained from vaginal delivery after uncomplicated pregnancy.

Placental tissue was cut into blocks that were immediately immersed in a solution of 4 per cent paraformaldehyde (w/v) in 0.1 M phosphate buffer, pH 7.4 for 6–8 h. To examine whether 4-HNE-reactive material was produced during tissue fixation and embedding, either 0.9 mmol/l azide, a myeloperoxidase inhibitor, or 20 µmol/l butylated hydroxytoluene and 2 nmol/l EDTA were added to the fixative used for some placentae (Boyd et al., 1989; Palinski et al., 1989, 1995; Hazell et al., 1996). After washing in phosphate buffer, the specimens

[c] To whom correspondence should be addressed.

0143–4004/97/040249+05 $12.00/0 © 1997 W. B. Saunders Company Ltd

were dehydrated through graded concentrations of ethanol, embedded in paraffin, and sectioned at 5–10 μm.

Immunohistochemistry

Staining protocol. Dewaxed sections were processed for the immunohistochemical demonstration of 4-HNE according to the indirect biotin-streptavidin immunoperoxidase technique (Hsu, Raine and Fanger, 1981). Briefly, the sections were incubated serially with the following solutions: (1) 0.3 per cent hydrogen peroxide for 30 min to remove endogenous peroxidase activity; (2) normal goat serum, diluted 1 : 20, for 30 min to reduce non-specific background staining; (3) mouse monoclonal antibodies to 4-HNE, at a range of dilutions (a dilution of 1 : 200 with overnight incubation at 4°C was found to be optimal); (4) pre-diluted biotinylated goat anti-mouse IgG (Super Sensitive kit BioGenex, San Ramon, USA), for 1 h at room temperature; (5) pre-diluted streptavidin-biotinylated peroxidase complexes (Super Sensitive kit, BioGenex, San Ramon, USA), for 1 h at room temperature; (6) 0.03 per cent, 3,3′diaminobenzidine tetrahydrochloride, to which hydrogen peroxide (0.02 per cent) was added just before use, for 5 min at room temperature. Each solution was prepared in 0.05 M Tris buffer, pH 7.4, containing 0.1 mol/l NaCl (0.15 M Tris-buffered saline) and between each step of the immunostaining procedure the sections were washed in the same buffer. Some sections were lightly counterstained with haematoxylin. The sections were finally dehydrated, mounted and observed in a Zeiss microscope equipped with Nomarski differential interference contrast device.

4-HNE-antibodies and specificity controls of the immunohistochemical reaction. Mouse monoclonal antibodies against 4-HNE (clones 1g4h7 and 1h4h12, subtypes IgG1 k) were produced using 4-hydroxynonenal coupled to bovine serum albumin. The epitope recognized by these clones was found to be 4-hydroxynonenal bound to hystidine. This epitope is recognized on any 4-HNE-modified protein so far tested, while native proteins do not show cross-reactivity. With regard to clone 1g4h7, less than 1 per cent cross-reactivity exists to 4-HNE-lysine or 4-HNE-cysteine. With regard to clone 1h4h12, 4-HNE-lysine is recognized with about 20 per cent cross-reactivity, while 4-HNE-cysteine shows 2 per cent cross-reactivity (data not shown). We have performed specificity tests, including adsorption of the antibodies with related and unrelated antigens, omission of the first layer and substitution of an inappropriate antiserum or a non-immune serum for the specific primary antibodies (Polak and Van Noorden, 1986). Immunostaining was completely prevented by absorption of HNE-antibodies with HNE coupled to albumin, whereas native albumin was uneffective in absorbing the antibodies (10 nmol in 1 ml antibodies diluted 1 : 200 in tris buffer).

As a further control of 4-HNE-immunostaining, we have used human carotid atherosclerotic lesions, which are known to contain 4-HNE-modified lipoproteins (Yla-Herttuala et al., 1989; Palinski et al., 1990; Rosenfeld et al., 1990), as positive control tissue.

RESULTS

Specific 4-HNE-immunoreactivity was observed in all placentae examined. The reaction appeared finely granular within the cytoplasm of trophoblast cells. In first trimester placentae, cytotrophoblast cells displayed stronger immunoreactivity compared with syncytiotrophoblast [Figure 1(A)]. In term placentae, where cytotrophoblast cells are greatly decreased in number, positive staining was observed in the syncytium [Figure 1(B)]. In the villous stroma of both first trimester and term placentae, mesenchymal cells and blood vessel endothelium were occasionally stained (Figure 2). No specific staining could be observed in control sections. Inclusion of antioxidants during tissue fixation did not result in any apparent differences in the staining (data not shown) when compared with tissues fixed using the routine procedure, indicating that the material recognized by 4-HNE-antibodies was not generated during sample preparation.

As a positive control of 4-HNE-immunocytochemical reaction, we revealed also 4-HNE-like immunoreactive material in atherosclerotic plaques (data not shown). The immunoreactivity pattern and the tissue distribution observed in our samples matched well with those reported in previous immunohistochemical studies (Yla-Herttuala et al., 1989; Palinski et al., 1990; Rosenfeld et al., 1990).

DISCUSSION

This study shows the occurrence and distribution of 4-HNE, a major propagation aldehydic product of lipid peroxidation, in normal human placenta. To date, the tissue distribution of 4-HNE and related molecules have been investigated only in pathological tissues, namely atherosclerotic lesions (Haberland, Fong and Cheng, 1988; Boyd et al., 1989; Yla-Herttuala et al., 1989; Palinski et al., 1990, 1995; Rosenfeld et al., 1990, 1991; Jurgens et al., 1993; Hazell et al., 1996). These investigations have provided convincing evidence that lipoperoxidation products actually occur in atherosclerotic plaques, thus providing further support to the hypothesis that oxidized lipoproteins might be involved in the pathogenesis of atherosclerosis (for a review see Witzum and Steinberg, 1991).

In general, increasing evidence exists that aldehydes generated during the process of lipid peroxidation are responsible for some of the effects associated with oxidative stress in cells and tissues (for a review see Esterbauer, Schaur and Zollner, 1991). In fact, unlike reactive free radicals, aldehydes are rather long lived and can attack intracellular targets that are distant from the initial free radical event. Therefore, it has been proposed that 4-hydroxyalkenals, such as 4-HNE, may be

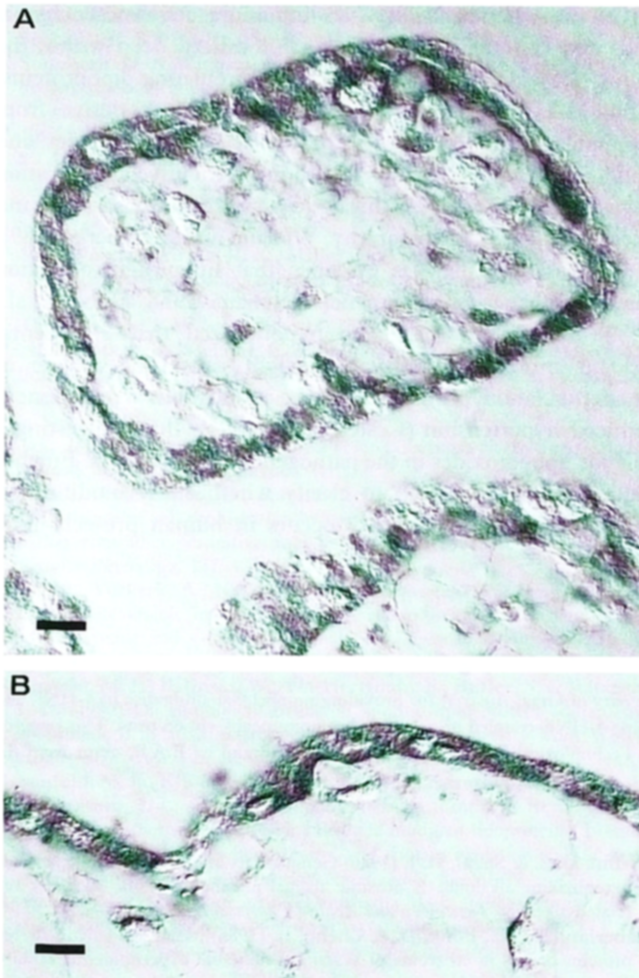

Figure 1. Immunohistochemical localization of 4-hydroxynonenal-immunoreactivity in (A) human first trimester and (B) term placentae. Immunostaining is detectable in trophoblast layers and some mesenchymal cells of he villous stroma. In first trimester placenta, the reaction is more intense in cytotrophoblast cells than in forming syncytium (A). In term placentae, where cytotrophoblast cells are reduced in number, the staining is visible in the syncytium (B). Indirect immunoperoxidase method. Bar=0.01 mm.

considered as second toxic messengers of the chain reactions that are initiated if polyunsaturated fatty acids are converted in lipid hydroperoxides (Esterbauer, Schaur and Zollner, 1991). In this connection, it must be considered that hydroxyalkenals are highly reactive molecules, that are able to react with SH and NH groups contained in aminoacids, proteins and nuclear nucleotides. Indeed, an extensive literature exists concerning the possible biological effects of hydroxyalkenals in cells and tissues. These include: (1) cytotoxicity (due to inhibition of DNA, RNA and protein synthesis, disturbance of calcium homeostasis, inhibition of respiration and glycolysis; Hauptlorenz et al., 1985; Griffin and Segal, 1986; Poot et al., 1988b); (2) modulation of adenylate cyclase activity and stimulation of phospholipase C (Dianzani, 1982; Rossi, Garramone and Dianzani, 1988; Rossi et al., 1990); (3) induction or reduction of gene transcription (such as c-myc; Barrera et al., 1987; Cajone, Salina and Benelli-Zazzera, 1989); and (4) cell

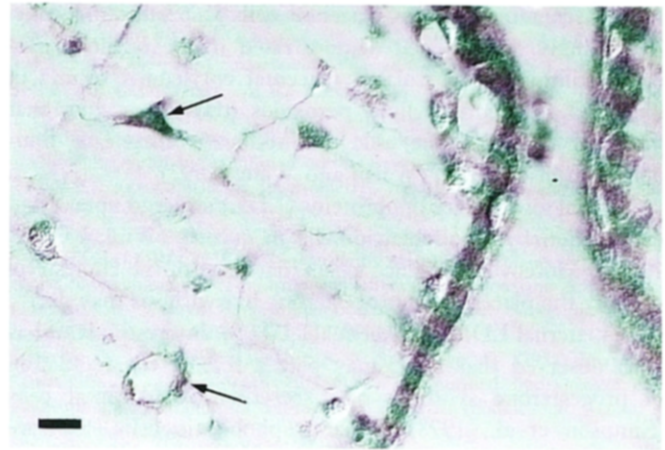

Figure 2. Immunohistochemical localization of 4-hydroxynonenal-immunoreactivity in human normal placenta. Beside trophoblast cells, some mesenchymal cells and endothelial cells of the villous stroma (Arrows) display positive staining. indirect immunoperoxidase method. Bar=0.01 mm.

growth inhibition (through inhibition of DNA polymerase system; Brambilla et al., 1986; Wawra et al., 1986; Poot et al., 1988a,b).

Although the formation of reactive aldehydes has been mostly studied in pathological or experimental systems, it has been shown that 4-HNE may also be produced physiologically, e.g. as a product from the peroxidation of liver microsomal lipids (Benedetti et al., 1979; Benedetti, Comporti and Esterbauer, 1980). This raises the possibility that physiological levels of hydroxyalkenals may have a role in normal cell metabolism.

To date, there is no information in the literature about the possible activity of 4-HNE and other hydroxyalkenals in placenta. The detection of 4-HNE-immunoreactivity in placenta raises the question whether lipoperoxidation products are produced locally in placental cells or represent exogenous products that derive from maternal blood flow. Scavenger receptor activity has been demonstrated in placental trophoblast cells and macrophages in vitro, thus suggesting that placenta may bind also oxidated lipoproteins that circulate in maternal blood flow (Malassiné et al., 1990; Bonet et al., 1995). In the case lipoperoxidation products including 4-HNE actually derive from maternal circulation, it may be speculated that trophoblast cells and mesenchymal cells displaying 4-HNE-immunoreactivity may play a protective role with regard to the diffusion of lipoperoxidation products from the mother toward the embryo.

However, very low levels of 4-HNE are detectable in plasma under physiological condition (Selley et al., 1989) and it has been reasoned that significant degree of lipid oxidative modification does not take place in plasma because of its high antioxidant content (Witzum and Steinberg, 1991). Thus, according to current hypothesis about oxidated lipoprotein formation (Witzum and Steinberg, 1991), it is presumable that lipid oxidative modifications occur mainly in tissue or cell microdomains that are sequestered from the many plasma antioxidants. In line with this view, it may be speculated that

4-HNE contained within placental cells is produced locally. Accordingly, it has been demonstrated that exogenous peroxide stimulation of isolated placental cotyledons results in increased secretion of lipid peroxides, thus suggesting that placental cells may be able to produce and release lipoperoxides also in vivo (Walsh and Wang, 1993).

Normal low density lipoproteins (LDL) undergo uptake and degradation by trophoblastic cells in culture (Winkel et al., 1980a). Moreover, evidence exists that most of the cholesterol used by the placenta for progesterone biosynthesis may derive from maternal LDL (Winkel et al., 1980b). Interestingly, it has been observed that LDL may play a role in the regulation of progesterone synthesis and secretion by placental cells (Simpson et al., 1978). Since trophoblastic cells, that we have found to be 4-HNE-immunoreactive, are provided by scavenger receptor, it may be hypothesized that LDL might be oxidized by trophoblast cells during blood traversal through the intervillous space.

On the other hand, 4-HNE-immunoreactive mesenchymal cells and endothelial cells, that are localized deep within the villus, should lack direct access to circulating lipoproteins. Thus, 4-HNE contained in villous cells may derive from trophoblast cells or may be produced by macrophages and endothelium that are able to produce oxidative modification via the lipoxygenase pathway (Sparrow, Parthasarathy and Steinberg, 1988; Parthasarathy, Wieland and Steinberg, 1989).

Interestingly, there is evidence that lipoprotein oxidation may be enhanced in pregnancy (Ishihara, 1978; Hubel et al., 1989). Recently it has been hypothesized that a placental oxidant–antioxidant imbalance might cause the release of lipoperoxidation products into the circulation in pregnancy-induced hypertension (Cester et al., 1994), thus suggesting a role for lipoperoxides in the pathogenesis of eclampsia. Further study will be addressed to clarify whether any modification of 4-HNE-immunoreactivity occurs in human pre-eclamptic placenta.

ACKNOWLEDGEMENTS

We are greatly indebted to Professor H. Esterbauer (Institute of Biochemistry, University of Graz, Austria) for providing monoclonal antibodies to 4-HNE and to Professor G. Bellomo (Institute of Pathology, University of Turin, Italy) for providing 4-HNE-coupled proteins used in immunoabsorption tests. This research was supported by grants from the Italian Ministry of University and Scientific and Technological Research (40 per cent funds) and by F.A.R. grant from the University of Pavia, Italy.

REFERENCES

Barrera, G., Martinottie, S., Fazio, V., Manzari, V., Paradisi, L., Parolam, M., Frati, L. & Dianzani, M. U. (1987) Inhibition of the expression of the c-myc oncogene in K 562 cells. *Toxicologic Pathology*, 15, 238–240.

Benedetti, A., Comporti, M. & Esterbauer, H. (1980) Identification of 4-hydroxynonenal as a cytotoxic originating from the peroxidation of liver microsomal lipids. *Biochimica et Biophysica Acta*, 620, 281–296.

Benedetti, A., Casini, A. F., Ferrali, M. & Comporti, M. (1979) Extraction and partial characterization of dialysis products originating from the peroxidation of liver microsomal lipids and inhibiting glucose 6-phosphatase activity. *Biochemical Pharmacology*, 28, 2009–2918.

Bonet, B., Chait, A., Gowan, A. M. & Knopp, R. H. (1985) Metabolism of modified LDL by cultured human placental cells. *Atherosclerosis*, 112, 125–136.

Boyd, H. C., Gowan, A. M., Wolfbauer, G. & Chait, A. (1989) Direct evidence for a protein recognized by a monoclonal antibody against oxidatively modified LDL in atherosclerotic lesions from Watanabe heritable hyperlipemic rabbit. *American Journal of Pathology*, 135, 815–826.

Brambilla, G., Sciaba, L., Faggin, P., Maura, A., Marinari, U. M., Ferro, M. & Esterbauer, H. (1986) Cytotoxicity, DNA fragmentation and sister-chromatid exchange hamster ovary cells exposed to the lipid peroxidation product 4-hydroxynonenal and homologous aldehydes. *Mutation Research*, 171, 169–176.

Cajone, F., Salina, M. & Benelli-Zazzera, A. (1989) 4-Hydroxynonenal induces a DNA-binding protein similar to the heat-shock factor. *Biochemistry Journal*, 262, 977–979.

Cester, N., Staffolani, R., Rabini, R. A., Magnanelli, R., Salvolini, E., Galassi, R., Mazzanti, L. & Romanini, C. (1994) Pregnancy induced hypertension. A role for peroxidation of microvillous plasma membranes. *Molecular and Cellular Biochemistry*, 131, 151–155.

Dianzani, M. U. (1982) Biochemical effects of saturated and unsaturated aldehydes. In *Free Radicals Lipid Peroxidation and Cancer* (Ed.) McBrian, D. C. H. & Slater, T. F. pp. 129–158. London: Academic Press.

Esterbauer, H., Schaur, R. J. & Zollner, H. (1991) Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radical Biology and Medicine*, 11, 81–128.

Griffin, D. S. & Segal, H. J. (1986) Genotoxicity and cytotoxicity of selected pyroolizidine alkaloids. A possible alkenal metabolite of the alkaloids and related alkenals. *Toxicology and Applied Pharmacology*, 86, 227–234.

Haberland, M. E., Fong, D. & Cheng, L. (1988) Malondialdehyde-altered proteins occurs in atheroma of Watanabe heritable hyperlipidemic rabbits. *Science*, 241, 215–241.

Hazell, L. J., Arnold, I., Flowers, D., Waeg, G., Malle, E. & Stocker, R. (1996) Presence of hypochlorite-modified proteins in human atherosclerotic lesions. *Journal of Clinical Investigations*, 97, 1535–1544.

Hauptlorenz, S., Esterbauer, H., Moll, W., Pumpel, R., Schauenstein, E. & Puschendorf, B. (1985) Effects of the lipid peroxidation product 4-hydroxynonenal and related aldehydes on proliferation and viability of cultured Ehrlich ascites tumor cells. *Biochemical Pharmacology*, 34, 3803–3809.

Hsu, S.-M., Raine, L. & Fanger, H. (1981) use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabelled antibody (PAP) procedures. *Journal of Histochemistry and Cytochemistry*, 29, 577–580.

Hubel, C. A., Roberts, J. M., Taylor, R. N., Musci, T. J., Rogers, G. M. & McLaughlin, M. K. (1985) Lipid peroxidation in pregnancy: new perspectives on preeclampsia. *American Journal of Obstetrics and Gynecology*, 161, 1025–1034.

Ishihara, M. (1978) Studies on lipoperoxide of normal pregnant women and patients with toxemia of pregnancy. *Clinica Chimica Acta*, 84, 1–9.

Jurgens, G., Chen, Q., Esterbauer, H., Mair, S., Ledinski, G. & Dinges, H. P. (1983) Immunostaining of human autopsy aortas with antibodies to modified apolipoprotein B and apoprotein(a). *Arteriosclerosis and Thrombosis*, 13, 1689–1699.

Knopp, R. H., Bergelin, R. O., Wahl, P. W. & Walden, C. E. (1985) Regulationships of infant birth size to maternal lipoproteins, apoprotein, fuel, hormones, clinical chemistries and body weight at 36 weeks gestation. *Diabetes*, 34, 71–77.

Knopp, R. H., Magee, M. S., Bonet, B. & Gomea-Coronado, D. (1991) Lipid metabolism in pregnancy In *Principles of Perinatal–Neonatal Metabolism*. (Ed.) Cowett, R. M. pp. 177–203. New York: Springer-Verlag.

Malassiné, A., Alsat, E., Besse, C., Rebourcet, R. & Cedard, L. (1990) Acetylated low density lipoprotein endocytosis by human syncytiotrophoblast in culture. *Placenta*, 11, 191–204.

Palinski, W., Rosenfeld, M. E., Yla-Herttuala, S., Gurtner, G. C., Socher, S. S., Butler, S. W., Parthasarathy, S., Carew, T. E., Steinberg, D. & Witzum, J. L. (1989) Low density lipoprotein undergo oxidative modifications in vivo. *Proceedings of the National Academy of Sciences of the USA*, **86**, 1372–1376.

Palinski, W., Yla-Herttuala, S., Rosenfeld, M. E., Butler, S. W., Socher, S. A., Parthasarathy, S., Curtiss, L. K. & Witztum, J. L. (1990) Antisera and monoclonal antibodies specific for epitopes generated during oxidative modification of low density lipoprotein. *Arteriosclerosis*, **10**, 325–335.

Palinski, W., Tangirala, R. K., Miller, E., Young, S. G. & Witzum, J. L. (1995) Increased autoantibody titers against epitopes of oxidized LDL in LDL receptor-deficient mice with increased atherosclerosis. *Arterioscleroris, Thrombosis and Vascular Biology*, **15**, 1569–1576.

Parthasarathy, S., Wieland, E. & Steinberg, D. (1989) Enzymatic modification of low density lipoprotein by purified lipoxygenase in the oxidative modification of low density lipoprotein. *Proceedings of the National Academy of Sciences of the USA*, **86**, 1946–1050.

Polak, J. M. & Van Noorden, S. (1986). *Immunocytochemistry. Modern Methods and Applications.* Bristol: Wright.

Poot, M., Esterbauer, H., Rabinovitch, P. S. & Hoehn, H. (1988a) Disturbance of cell proliferation by two model compounds of lipid peroxidation contradicts causative role in proliferative senescence. *Journal of Cellular Physiology*, **137**, 421–429.

Poot, M., Verkerk, A., Koster, J. F., Esterbauer, H. & Jongkind, J. F. (1988b). Reversible inhibition of DNA and protein synthesis by cumene hydroperoxide and 4-hydroxynonenal. *Mechanism of Ageing and Development*, **43**, 1–9.

Rosenfeld, M. E., Palinski, W., Yla-Herttuala, S., Butler, S. & Witztum, J. L. (1990) Distribution of oxidation specific lipid-protein adducts and apoprotein B in atherosclerotic lesions of varying severity from WHHL rabbits. *Arteriosclerosis*, **10**, 336–349.

Rosenfeld, M. E., Khoo, J. C., Miller, E., Parthasarathy, S., Palinski, W. & Witztum, J. L. (1991) Macrophage-derived foam cells freshly isolated from rabbit atherosclerotic lesions degrade modified lipoproteins, promote oxidation of low-density lipoproteins, and contain oxidation-specific lipid-protein adducts. *Journal of Clinical Investigation*, **87**, 90–99.

Rossi, M. A., Garramone, A. & Dianzani, M. U. (1988) Stimulation of phospholipase C activity by 4-hydroxynonenal: influence of GTP and calcium concentration. *International Journal of Tissue Reactions*, **10**, 321–325.

Rossi, M. A., Fidale, F., Garramone, A., Esterbauer, H. & Dianzani, M. U. (1990) Effect of 4-hydroxyalkenals on hepatic phosphatidylinositol-4,5-biphospholipase C. *Biochemical Pharmacology*, **39**, 1715–1719.

Selley, M. L., Bartlett, M. R., McGuiness, J. A., Hapel, A. J., Ardlie, N. G. & Lacey, M. J. (1989) Determination of the lipid peroxidation product tans-4-nonenal in biological samples by high performance liquid chromatography and combined capillary gas chromatography/negative ion chemical ionization mass spectrometry. *Journal of Chromatography*, **488**, 329–340.

Simpson, E. R., Porter, J. C., Milewich, L., Biheimer, D. W. & MacDonald, P. C. (1978) Regulation by plasma lipoproteins of progesterone biosynthesis and 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in cultured human choriocarcinoma cells. *Journal of Clinical Endocrinology and Metabolism*, **47**, 1099–1105.

Sparrow, C. P., Parthasarathy, S. & Steinberg, D. (1988) Enzymatic modification of low density lipoprotein by purified lipoxygenase plus phospholipase A2 mimics cell-mediated oxidative modification. *Journal of Lipid Research*, **29**, 745–753.

Walsh, S. W. & Wang, Y. (1993) Secretion of lipid peroxides by the human placenta. *American Journal of Obstetrics and Gynecology*, **169**, 1462–1466.

Wawra, E., Zollner, H., Schaur, R. J., Tillian, H. M. & Schauenstein, E. (1986) The inhibitory effect of 4-hydroxynonenal on DNA-polymerases alpha and beta from rat liver and rapidly dividing Yoshida ascites hepatoma *Cell Biochemistry and Function*, **4**, 31–36.

Winkel, C. A., Gilmore, J., MacDonald, P. C. & Simpson, E. R. (1980a) Uptake and degradation of lipoproteins by human trophoblastic cells in primary culture. *Endocrinology*, **107**, 1892–1898.

Winkel, C. A., Snyder, J. M., MacDonald, P. C. & Simpson, E. R. (1980b) Regulation of cholesterol and progesterone synthesis in human placental cells in culture by serum lipoproteins. *Endocrinology*, **106**, 1054–1060.

Witztum, J. L. & Steinberg, D. (1991) Role of oxidized low density lipoprotein in atherogenesis. *Journal of Clinical Investigation*, **188**, 1765–1792.

Yla-Herttuala, S., Palinski, W., Rosenfeld, M. E., Parthasarathy, S., Carew, T. E., Butler, S., Witztum, J. L. & Steinberg, D. (1989) Evidence for the presence of oxidatively modified low density lipoprotein in atherosclerotic lesions of rabbit and man. *Journal of Clinical Investigation*, **84**, 1086–1097.