LIPOPROTEINS AND ATHEROSCLEROSIS

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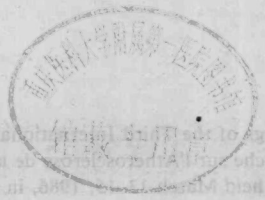

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PREFACE

Atherosclerosis which accounts in Western Europe for more than 40 % of deaths, is a generalized disease that develops slowly and is symptomless until lesions have become sufficiently severe to cause myocardial or cerebral infarction.

Research on specific and precocious markers of atherosclerosis and the development of non invasive techniques for their early detection represent major challenges in biomedical field.

We hope that this volume of edited papers, a consequence of the third international colloquium on atherosclerosis, conducted at the University of Brussels, Belgium through the support of the "Fondation de Recherche sur l'Athérosclérose" will contribute to this goal.

Among the topics discussed the major ones were the mechanism of action of lipolytic enzymes, the deficiency or dysregulation of cellular receptors, the genetic deficiencies of apolipoproteins, and the panoply of external factors as diet, physical exercise, drugs, which modify the lipoprotein metabolism. Special interest was also devoted to potent techniques as kinetic analysis of metabolic tracers and use of monoclonal antibodies. Their contribution to the detection and treatment of atherosclerosis will be obviously essential in the future.

C.L. Malmendier

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HETEROGENEITY OF APOLIPOPROTEIN B CONTAINING LIPOPROTEINS

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Apolipoprotein (apo) B which is a major protein component of low density lipoproteins (LDL), very low density lipoproteins (VLDL) and chylomicrons plays an important role in the transport of lipids. Clinically, LDL apo B has been shown to be a risk factor for coronary artery disease which is independent of LDL cholesterol[1]. Apo B is known to be chemically and metabolically heterogeneous. The major species present in LDL and VLDL, apo B100, which is synthesized in the liver has an apparent molecular weight of 550,000, whereas that of chylomicrons, apo $B48_2$ is of intestinal origin and has an apparent molecular weight of 264,000[2]. Apo B100 and apo B48 are clearly related immunologically and are probably products of the same gene. Like apo E, apo B100 is a ligand for the LDL receptor and is therefore critical for the maintenance of cholesterol homeostasis. While apo B is relatively abundant and easily isolated free of other proteins, its insolubility, suseptibility to proteolysis and tendancy to aggregate have provided serious technical obstacles to its characterization[3,4]. It is only recently that extensive knowledge of apo B primary structure has become available through the sequencing of apo B cDNA.

Apo B containing lipoproteins (LpB) are heterogeneous, varying from large triglyceride (TG)-rich particles such as chylomicrons and VLDL to much smaller cholesterol-rich LDL. In normal humans, almost all LDL originates from VLDL particles of hepatic origin which undergo intravascular lipolysis and exchanges of lipid and proteins with other lipoprotein classes. This progessive transformation of VLDL to LDL generates a spectrum of intermediate particles of differing diameter, hydrated density and chemical composition. Heterogeneity in the chemical and physical properties of LpB particles is also present within the LDL density class. Further heterogeneity within the LDL class can be introduced by disease. In familial hypercholesterolemia, there is the appearance of a cholesterol-rich subfraction of LDL[5,6], whereas hyperapobetalipoproteinemia[1] and familial combined hyperlipoproteinemia are associated with an accumulation of small LDL particles which are relatively depleted of cholesterol and enriched in protein.

There is now considerable evidence to indicate that this physical and chemical heterogeneity of LpB can introduce heterogeneity in the conformation of apo B. It has been shown that as VLDL undergoes lipolysis, there is a progressive increase in the immunoreactivity of the

apo B with certain anti-apo B polyclonal antisera[7] and monoclonal antibodies (Mabs)[8] and that this can be due to both changes in apparent antibody affinity and in epitope expression (8). This increase in immunoreactivity is accompagnied by an increase in the ability of the apo B to react with the LDL receptor[7,9]. In large TG-rich VLDL, apo E is the principal ligand which is recognized by the LDL receptor and as TG are removed from the particle, either in vivo or in vitro, the apo B component of the binding becomes more important[9-11]. It would therefore appear that as VLDL are converted to LDL, the changes in the particle structure result in either an unmasking of specific regions of apo B or a change in its conformation such that interactions with both antibody and cell surface receptors are facilitated.

The chemical and physical heterogeneity within the LDL class is also manifested in an immunochemical heterogeneity. We have shown that the immunoreactivity of certain antigenic determinants defined by anti-apo B_{LDL} Mabs varies in a predictable manner as a function of the cholesterol to protein ratio of the particles[12]. The larger more buoyant cholesterol-rich LDL were more immunoreactive than were the smaller less buoyant cholesterol-depleted LDL. By a comparison of results obtained in a competitive radioimmunometric assay[12] with those obtained by immunoprecipitation (unpublished results), we have concluded that the reported immunochemical heterogeneity reflects primarily differences in antibody affinity rather than heterogeneity in antigenic expression. As others have reported (see above), we have observed that the immunoreactivity of certain of our anti-apo B_{LDL} Mabs with VLDL increases as the VLDL undergoes lipolysis (unpublished results). As was the case for the LDL, the differences were more evident in the competitive radioimmunometric assay than in immunoprecipitation experiments. Thus, it would appear that the epitopes defined by certain of the apo B_{LDL} Mabs have an optimal conformation when they are present on cholesterol-rich LDL. The Mabs react with lower affinity when the apo B is in the form of either larger TG-rich VLDL or smaller cholesterol-depleted LDL. It is not known whether particle diameter or particle composition plays the more important role in modulating apo B conformation.

We have now localized the epitopes of those Mabs whose immunoreactivity varies with the LDL cholesterol to protein ratio to the middle one third of the apo B molecule (unpublished results). This may indicate that changes in the physical and chemical properties of LDL may alter the conformation of relatively large regions of the apo B molecule as opposed to that of individual isolated epitopes distributed randomly throughout apo B. Two of these Mabs are capable of inhibiting the binding of LDL to the LDL receptor[13]. As it is probable that the ability of these Mabs to block receptor binding reflects the proximity of their corresponding epitopes to the receptor-binding domaine of apo B, it may indicate that the conformation of the receptor-binding domaine itself may be suseptible to modulation by the changes in the LDL composition which, in turn, could influence LDL metabolism. In support of this, it has been demonstrated that the less buoyant cholesterol-poor LDL have a lower fractional catabolic rate than the larger cholesterol-rich LDL[14]. Furthermore, Kleinman et al.[15] have demonstrated that the small LDL characteristic of certain hypertriglyceridemic subjects are replaced by LDL of normal diameter following treatment with bezofibrate. While the small LDL present before treatment of the hypertriglyceridemia are poorly recognized by the LDL receptor, the larger LDL which appear in response to bezofibrate are normally taken up by cultured human fibroblasts.

We have found that most epitopes recognized by anti-apo B_{LDL} Mabs require lipid for their expression[16]. Moreover, individual epitopes differ in the minimal lipid ennvironment necessary for their expression. Thus, while only one of the previously characterized anti-apo B_{LDL} Mabs reacted with delipided resolubilized apo B (apo B_{sol}), reactivity of the apo B_{sol} with another Mab (2D8) could be regenerated by reincorporation of the apo B into phospholipid-cholesterol liposomes. Other anti-apo B_{LDL} Mabs reacted only with apo B_{sol} which had been reincorporated into phospholipid-cholesteryl ester microemulsions. Thus, in these experiments, three classes of apo B epitopes were defined; those which were independent of lipid, those which required the presence of amphipathic lipids and those which required both amphipathic lipids and a neutral cholesteryl ester core. It is perhaps significant that the anti-apo B_{LDL} Mabs with the most stringent lipid requirements were those which react with epitopes close to the receptor binding domaine of apo B. This would again suggest the lipid-dependent conformation of this region of apo B. Recently, we have prepared and characterized Mabs against apo B_{sol}[17]. Most of the anti-apo B_{sol} Mabs react preferentially with apo B_{sol} and, in contrast to the anti-apo B_{LDL} Mabs, reincorporation of the apo B_{sol} into lipid vesicles generally resulted in a loss of immunoreactivity with the anti-apo B_{sol} Mabs. Thus, lipids can both negatively and positively modulate expression of apo B epitopes. Furthermore, the reported temperature-dependent variation in the apparent affinity of certain anti-apo B_{LDL} Mabs for LDL may reflect the dependence of apo B conformation on the physical state of the associated lipids[18].

It would therefore appear that the conformation of apo B is highly dependent on the physical and chemical properties of the lipoproteins in which it is found. Moreover, this apparent modulation of apo B conformation may have consequences on the biological function of the LpB such as the interaction with cell surface receptors. It has been suggested that the small cholesterol-poor LDL subfraction which is prominant in subjects with hyperapobetalipoproteinemia may be especially atherogenic (1). As described above, Mabs detect immunochemical differences amongst the LDL subfractions. Therefore antibodies which have been prepared against and react preferentially with this cholesterol-depleted LDL subfraction may prove to be particularly discriminating predictors of atherosclerosis.

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SEPARATION AND IDENTIFICATION OF APO-B-CONTAINING LIPOPROTEIN PARTICLES IN NORMOLIPIDEMIC SUBJECTS AND PATIENTS WITH HYPERLIPOPROTEINEMIAS

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INTRODUCTION

The operationally-defined plasma lipoproteins have provided the theoretical basis for most studies and current views on the mechanism of lipid transport [1,2]. The popularity of these classification systems has been enhanced by clinical studies which have related certain derangements of lipid transport to particular density classes or electrophoretic patterns [2,3]. However, the discovery of a number of apolipoproteins [1,4] and the detection of marked protein heterogeneity of lipoprotein density classes and electrophoretic bands [4,5] have become incompatible with the view that operationally-defined lipoproteins represent the fundamental chemical and metabolic entities of lipid transport system. Results from laboratories have shown that major density classes consist of several distinct lipoprotein families or particles rather than single lipid-protein complexes [4-9]. Based on these and similar findings showing that lipoprotein density classes consist of distinct lipoprotein particles of similar hydrated densities but different apolipoprotein composition, we have proposed that apolipoproteins be used as specific markers for the identification of lipoprotein particles and as a new means for the classification of plasma lipoproteins [10,11]. According to this proposal, lipoprotein families or particles which contain a single apolipoprotein are called simple lipoproteins and those which contain two or more apolipoproteins are referred to as complex lipoproteins [11]. The nomenclature of lipoprotein particles is based on the ABC nomenclature of apolipoproteins [10] in that lipoprotein particles are named after their constitutive apolipoproteins. For example, lipoprotein particles which only contain ApoB as their protein moiety are called lipoprotein B (LP-B), while lipoprotein particles which contain apolipoproteins B, C and E are named lipoprotein B:C:E (LP-B:C:E).

Although the characterization of plasma lipoproteins under normal and pathophysiological conditions rests on the quantitative determination of individual lipid and, more recently, apolipoprotein constituents, such measurements encompass the lipid and/or apolipoprotein mass of all plasma or density class lipoproteins rather than individual simple and complex lipoprotein particles. If one assumes that simple and complex lipoprotein particles represent the fundamental physicochemical and functional entities of the lipid transport, then the proper functioning of this system depends on certain characteristic concentrations of these lipoprotein particles. Any alterations of this physiological process ought to result in their