Regression of Atherosclerotic Lesions

M. Rene Malinow and Victor Blaton

The Advanced Study Institute on Regression of Atherosclerotic Lesions, sponsored by the North Atlantic Treaty Organization, was held at Maratea, Italy, September 11-23, 1983. The director was M. Rene Malinow and the codirector was Victor Blaton. Fifteen leading research scientists and more than 30 students from Europe, North America, the Middle East, and Africa attended. The institute was designed to foster discussion of experimental data on the regression of atherosclerosis in animals and evidence of a similar phenomenon in humans. Experts in the field gave presentations on a wide variety of topics, and many students gave short communications. The program allowed ample time for discussion, for one-on-one conversations, and for maximum interaction among participants.

Introduction: Arterial Wall Model

M. Rene Malinow opened the meeting by describing a model of the arterial wall in which regression occurs when the outward flows exceed the inward flows in the transport and transformation of atheromatous materials. The model involves a series of plates that may account for the relative independence of adjoining segments, and thus allow for simultaneous progression and regression in different arterial regions. Although regression of atherosclerosis has been demonstrated in animals, its existence in humans has been inferred from contrast angiographic studies in which the arterial walls were not visible in the absence of calcification. It is likely that regression in humans involves not only changes in the arterial wall — which may be similar to those in animals — but also reabsorption or recanalization of thrombi, or other poorly understood phenomena.

Regression of Atherosclerosis in Animals

Components of Arteriosclerotic Plaques

Robert W. Wissler (Chicago, Illinois) stressed features of the components of atherosclerotic plaques, including the modified smooth muscle cell (the main cell type in the human plaque); the apolipoprotein B-containing plasma lipoproteins, which are the main sources of lipids; the receptors for apolipoproteins on cell surfaces and their role in regulating intracellular metabolism; the stimulation of smooth muscle cell proliferation by growth factors from plasma and cells (platelets, macrophages, and endothelial cells); the functions of lysosomal enzymes; the importance of endothelial injury; the contributions of macrophages to the cell population; the importance of certain lipoproteins in the development and prevention of plaques; and the modulation of plaque morphology by certain types of dietary fat.

He also reported on a series of experiments in rhesus macaques wherein atherosclerosis caused by eating large amounts of cholesterol was made to regress by consumption of foods low in fat and cholesterol or the administration of bile acid sequestrants with or without probucol.

Comparative Pathology

Thomas B. Clarkson (Winston-Salem, North Carolina) described the comparative pathology of nonhuman primate atherosclerosis. Research on various species of nonhuman primates has demonstrated the suitability of certain ones for the study of particular problems. For instance, older cebus monkeys are more susceptible to atherosclerosis than younger ones, and they have large and complicated plaques of the carotid bifurcation. Squirrel monkeys are genetically predisposed to be hypo- and hyperresponsive to dietary cholesterol; they show lesion complications in the abdominal aorta and exacerbation of the atherosclerotic lesions by experimental diabetes mellitus independently of cholesterolemia; some data on squirrel monkeys suggest a relationship between herpes infection and the development of atherosclerosis.

Rhesus macaques are a valuable model since the pathogenesis of their atherosclerosis resembles that of humans. Cynomolgus macaques are probably the most useful of all macaques in atherosclerosis research because their atherosclerotic lesions become complicated, there are male-female differences in lesion extent, coronary artery atherosclerosis is influenced by personality type and social situation, and myocardial infarction occurs with a relatively high frequency (about 1 in 300 animals at
risk). Stump-tailed macaques seem useful for studies on the relationships among obesity, plasma lipids, hypertension, and atherosclerosis. African green monkeys seem particularly useful for studies on lipoprotein changes induced by diet, which bear a striking resemblance to the changes observed in humans eating food containing cholesterol and saturated fat.

**Experiment with Rhesus Macaques**

A regression experiment initiated in 1972 on male rhesus macaques was also discussed by Dr. Clarkson. Atherosclerosis was induced by either 19 months or 38 months on a high fat, high cholesterol diet. During the next 4 years, the monkeys were maintained on diets that kept their cholesterolemia at either 200 mg/dl or 300 mg/dl, and groups of monkeys were killed after 2 or 4 years on these diets. The potentials for regression were similar in the animals whose atherosclerosis was induced for 19 and for 38 months; however, regression was more apparent in the monkeys with cholesterolemia around 200 mg/dl. An interesting observation was the increase in artery diameter associated with the size of the intimal lesions. Furthermore, atherosclerosis regressed in the majority of the hyporesponder (HO) monkeys with cholesterolemia around 300 mg/dl, whereas it progressed in the majority of the hyperresponders (HR) at that level of plasma cholesterol.

**Experiment with Cynomolgus Macaques**

Dr. Malinow discussed the suitability of cynomolgus macaques for regression studies. Lipoprotein levels were observed in HO and HR monkeys on a chow diet and on semipurified diets high in saturated fat, with or without added cholesterol. The HO monkeys had lower levels of plasma cholesterol than the HR monkeys, even when they ate monkey chow, which is almost free of cholesterol. Eating food rich in saturated fat decreased high density lipoprotein-2 (HDL₂) and increased HDL₃ concentrations in both types of monkeys. The addition of cholesterol to the diet increased very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), and low density lipoprotein (LDL) more in the HR than in the HO monkeys. Although HDL₂ increased in the HO monkeys, HDL₃ and HDL₄ decreased in the HR monkeys.

Cynomolgus macaques may exhibit minimal or advanced atherosclerosis at similar plasma levels; the former are called hyporeactors (R⁻) and the latter hyperreactors (R⁺). This phenomenon was first observed in rabbits. Cholesterol-fed cynomolgus macaques show fatty cellular infiltration of the media that correlates positively with the extent of intimal involvement. The medial infiltration decreases during regression, so it is unlikely that an immunologic mechanism is involved.

Cynomolgus macaques constitute an excellent model for the study of regression under the following conditions: 1) atherosclerosis is induced during a 6-month intake of semipurified food containing 1.2 mg of cholesterol/Kcal; 2) animals are allocated to experimental groups by a strictly randomized procedure; 3) there are sufficient numbers of animals in each group. The effects of an alfalfa meal extract, operationally named *alfalfa saponins*, were discussed. These saponins reduced cholesterolemia and induced regression of aortic and coronary atherosclerosis without signs of toxicity.

**Diet-Induced Changes in Lipids**

Victor Blaton (Bruges, Belgium) described in detail the plasma lipoprotein lipids of nonhuman primates and the changes induced by different diets. Detailed analyses of lipids and fatty acids of plasma lipoproteins were designed to relate the diet-induced changes in type and composition of plasma lipoproteins to arterial lesions in nonhuman primates. Similar studies on the effects of diets rich in polynsaturated triglycerides and polyunsaturated phosphatidyl-choline on human and nonhuman plasma lipoproteins were discussed. Finally, changes in the composition of plasma lipoproteins were correlated with the regression of atherosclerosis in rhesus macaques on diets rich in polyunsaturated fatty acids and containing cholestyramine.

**SEM and TEM Studies**

Giorgio Weber (Siena, Italy) described a scanning electron microscopic and transmission electron microscopic study of the regression of arterial lesions in cholesterol-fed rhesus macaques, cynomolgus macaques, and rabbits. Lesions regressed in monkeys when plasma cholesterol levels decreased due to dietary interventions or eating of cholestyramine. In rabbits, regression was observed after partial ileal bypass, and was associated with a large drop in cholesterolemia. Dr. Weber also described the glyocalyx stained with concanavalin A in the endothelial surfaces of adult rabbit aortas and coronary, carotid, and femoral arteries. No glyocalyx was observed, however, in the intracranial arteries. He indicated that its absence might be related to a lack of lipoprotein lipase activity which would not allow chylomicron remnants to be trapped, making the cerebrovascular vessels of rabbits resistant to atherosclerosis.

**No Degradation of Ether Analogues**

Olga Stein, Yezechiel Stein, and Gideon Halperin (Jerusalem, Israel) found that ether analogues of cholesterol esters are not degraded in vitro or in vivo. The influx of these ethers into the arterial wall in cholesterol-fed rabbits correlates well with the mass of cholesterol ester in atherosclerotic intima and media of the aorta. Since these ether analogues are not degraded, their recovery in the aorta of a rabbit undergoing regression of atherosclerosis could be useful in estimating the amount of cholesteryl ester present before the onset of regression.
Interactions Between Lipids and Macromolecules

Ladislas Robert, Jean Chaudiere, and Bernard Jacotot (Creteil, France) described the interactions between lipids and intercellular matrix macromolecules of the arterial wall, such as elastin, collagens, proteoglycans, and glycoproteins. Elastin isolated from atherosclerotic human aortas is especially rich in cholesterol esters and free fatty acids. These interactions decrease elasticity and increase susceptibility to elastolysis. The calcium ion enhances the interaction of elastin with lipids. In rabbits maintained on a 1% cholesterol diet and then on a cholesterol-free diet containing 5% oil, elastolysis and intimal proliferation appeared to be independent.

Four different triglycerides were tested during this 3-month, postcholesterol regression period: butter, olive oil, sunflower oil, and primrose oil. Only sunflower oil slowed down or stopped the progression of atherosclerosis; butter, olive oil, and primrose oil were ineffective or even increased the lesions. The strongest elastolysis was observed with butter and the strongest intimal proliferation with primrose oil, which is rich in γ-linolenic acid.

The method used at the Paris-XII University for clinical pharmacologic studies was also discussed. The usefulness of skin biopsies for the determination (by a computerized quantitative histochemical procedure) of elastolysis and LDL deposition in the dermal intercellular matrix was stressed.

Studies of Human Atherosclerosis

Apolipoprotein B-Containing Lipoproteins

Kenneth W. Walton (Birmingham, England) discussed human atherosclerotic lesions characterized at all stages of their development by the presence of apolipoprotein B-containing lipoproteins, fibrinogen, and fibronectin. He also illustrated the interaction of apolipoprotein B-containing lipoproteins with the fibers and interstitial matrix of the intimal gel, which gives rise to extracellular deposits, and contrasted these findings with the receptor-mediated and scavenger pathways of intracellular lipid deposits.

Role of Plasma Lipoproteins

Gerd Assmann (Münster, West Germany) discussed the role of plasma lipoproteins in the pathogenesis of atherosclerotic lesions. He emphasized the distribution of lipoprotein apoproteins in lipoproteins; the structure-function relationships of apolipoproteins; the surface receptors that control lipoprotein metabolism; the genetic disorders of lipoprotein metabolism and their possible roles in atherogenesis; and the possible early diagnosis of coronary heart disease based on the epidemiologic study of LDL, HDL, and certain apolipoproteins.

Composition of Interstitial Fluid

Elspeth B. Smith (Aberdeen, Scotland) presented her findings on the composition of interstitial fluid in the intima. The observation that the LDL concentration was twice that in plasma suggested that LDL is trapped between the endothelium and internal elastic lamina. The ratio of LDL to other plasma macromolecules in interstitial fluid was greater than in intimal tissue; this finding does not support the hypothesis of specific reversible sequestration of LDL by components of the connective tissue matrix.

In early proliferative (gelatinous) lesions, there was a large volume of interstitial fluid containing a relatively small concentration of LDL — 80% to 200% of the concentration in adjacent normal intima. However, in more mature lesions, water appeared to shift out of the interstitial fluid compartment leaving a small amount of interstitial fluid containing large concentrations of LDL. This phenomenon may be a major factor in the deposition of extracellular lipid in fibrous plaques.

By contrast, in fatty streaks containing numerous fat-filled cells, the LDL concentration was small, and in some samples the interstitial fluid was totally depleted of LDL. These observations suggested that the fat-filled cells internalize and degrade LDL faster than it enters the intima.

Comparison of Lesions in Humans and Monkeys

Herbert C. Stary (New Orleans, Louisiana) described the structure of the coronary arteries and aortas in 300 persons who died of accidental causes between full-term birth and 29 years, and in a small sample of older persons. The lesions were compared to those found in the coronary arteries of the following: 1) rhesus macaques fed high-cholesterol foods for 1 to 24 months; 2) rhesus macaques fed high-cholesterol foods for 3 or 24 months, and then fed low cholesterol foods for 0.5 to 24 months; 3) patas monkeys on a high cholesterol diet for 5 or 10 months; and 4) cynomolgus macaques on a high cholesterol diet for 6 months. Because of the thoroughness of the electron microscopic observations, the fact that the arteries were pressure-perfused, and the extensive number of human subjects included in the survey, this data will probably have an important impact on knowledge of human atherosclerosis and the comparative anatomy of nonhuman primates.

Issues in Human Studies

Richard J. Havel (San Francisco, California) described a study being carried out at the Cardiovascular Research Institute of the University of California, San Francisco. Patients with heterozygous familial hypercholesterolemia treated by the combination of bile acid-binding resin and nicotinic acid are evaluated by serial coronary and femoral angiography. A comparison of this data with information on placebo-treated controls is designed to establish whether atherosclerosis can be modified by a significant lowering of the plasma LDL level. He commented on several important issues in human studies including
sample size, patient motivation and program adher-
ence, problems associated with assigning patients at
high risk to placebo preparations, acceptance of two
angiographic examinations, adherence to diet and
medication, and the need for patients to visit the
clinic at monthly intervals for 2 years. A high rate of
patient compliance requires a highly motivated and
well-trained team of health professionals.

Drug Therapy to Lower Lipid Levels

David H. Blankenhorn and Stanley P. Azen (Los
Angeles, California) described a therapeutic regi-
men to decrease lipid levels in humans in order to
lessen atherosclerosis. The Computer Estimated
Atherosclerosis Index permits measurement of the
rate of change in atherosclerotic lesions in the fem-
oral artery, when two angiograms are taken. The
results in a group of 180 nonsmoking men, aged 40
to 59, who have had coronary bypass operations,
randomly allocated to colestitol-niacin therapy or to
a placebo, will establish whether a fall in plasma LDL
diabetes and a rise in plasma HDL levels can induce
regression of femoral atherosclerosis. Observation
of the coronary and carotid arteries will provide infor-
mation on the rates of change so intervention strat-
ies can be designed. The investigators reported the
status of nine studies currently in progress, as well
as a cholesterol-lowering study in progress at the
University of Southern California. The data gathered
thus far on 66 controls and 71 drug-treated patients
have shown a significant reduction in the drug-treat-
ed group in cholesterol (25%), LDL cholesterol
(41%), and triglycerides (15%); there has been an
increase in HDL cholesterol (34%) and significant
increases in serum uric acid, alkaline phosphatase,
and lactic dehydrogenase. Dr. Azen also comment-
ed on statistical issues in human studies, including
the allocation ratios, the inappropriateness of a cros-
sover design, the variability in human evaluation of
stenosis, the possible error in computer estimation of
atherosclerosis, and the problems with sample size.

Angiographic Evidence of Regression in
Coronary Arteries

B. Greg Brown, Edward L. Bolson, and Howard T.
Dodge (Seattle, Washington) reported angiographic
evidence of regression of coronary atherosclerosis
in humans. The 47 prospectively enrolled patients
underwent elective angiography 18 months after an
initial, clinically indicated coronary arteriogram. The
642 coronary segments were serially measured with
computer assistance. Of the lesions, 12% pro-
gressed and 4% regressed, i.e., decreased in per-
cent stenosis by at least 10.2%. The probability of
lesion change was affected by the initial lesion se-
verity and, to a lesser extent, by the patient’s age and
the extent of hyperlipidemia. The average stenosis
change in the progression group was 18%; in the
regression group it was 14%. Although regression of
coronary atherosclerosis was infrequent in this un-
treated sample, its occurrence provides encour-
agement that it may be induced by suitable intervention.

Angiographic Evidence of Regression in
Femoral Arteries

Anders G. Olsson (Stockholm, Sweden) reported
his ongoing studies on the regression of femoral
atherosclerosis in asymptomatic men with hyperlipo-
proteinaemia. The 60 patients — 20 each with type
IIA, IIB, or IV hyperlipoproteinemia — were assigned
to a diet-placebo regimen or to drug treatment (diet
plus nicotinic acid and fenofibrate). This study will be
completed by the end of 1985; Dr. Olsson discussed
here eight pilot cases in which femoral angiography
was performed four times over a 2-year period. Re-
gression of atherosclerosis was observed in six men
and correlated with a drop in plasma LDL cholesterol
levels.

Anatomical Evidence of Regression

M. Rene Malinow reported anatomical evidence of
regression of atherosclerosis in humans. A survey of
several studies suggested that the extent of ather-
sclerosis was reduced in certain populations during
the lean years of World Wars I and II, and that it is
reduced during wasting diseases. However, selec-
tion bias, as well as other confounding variables,
makes interpretation of these findings difficult. Even
if present, regression of atherosclerosis could not be
separated from arrest of progression. Many, but not
all, investigators agree that patients with cancer
show less extensive atherosclerosis than patients
without cancer. A large series of cancer and non-
cancer patients was analyzed, and the findings
were consistent with the hypothesis that processes
occurring during the terminal stages of cancer are
associated with the arrest of progression and with
the regression of atherosclerosis. However, be-
cause of the uncertainty of retrospective postmortem
data, these conclusions are tentative.

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