European Association for the Study of Diabetes (EASD) 2001 Meeting

ZACHARY T. BLOOMGARDEN, MD

This report is on the 37th Annual Meeting of the European Association for the Study of Diabetes (EASD), held in Glasgow, U.K., from 9 to 13 September 2001. It covers such topics as the primary prevention of type 2 diabetes and potential therapeutic strategies for diabetic neuropathy.

Peroxisome proliferator–activated receptor-γ and human metabolic disease

At the Robert Turner Memorial Lecture opening the 37th Annual Meeting of the European Association for the Study of Diabetes (EASD), Stephen O’Rahilly (Cambridge, U.K.) discussed the relationship between peroxisome proliferator–activated receptor (PPAR)-γ and human metabolic disease. He tried to “get some of the flavor of Robert [Turner]” in his analysis of “extreme human phenotypes” having abnormality of the PPAR-γ gene, pointing out that “experiments of nature” are serious diseases with high morbidity and mortality that can illuminate normal biology and act as models for common diseases. He noted that similar studies of individuals who lack estrogen receptor or IGF-1 have illuminated other more common clinical syndromes.

His Genetics of Obesity program has enrolled children with prepubertal onset and weight >3 SDs. Studies of two related children with severe obesity lacking functional leptin have shown that deficiency of this hormone in humans causes pathology similar to that described in mice. Two other pedigrees with the same mutation were subsequently discovered. Treatment of four leptin-deficient patients with recombinant human leptin markedly decreased energy intake and lowered fat mass. These children had decreased interferon (IFN)-γ production by T-cells that was reversed by leptin treatment. Individuals without leptin fail to enter puberty. Normal age-appropriate gonadotropin response is seen with replacement treatment. O’Rahilly speculated that the physiologic role of leptin may be particularly important when levels are very low and that the main function of leptin may be to signal the transition between the fed and starved states. Heterozygotes from these families may have an abnormal relationship between BMI and leptin, showing mild obesity with disproportionate increase in fat, suggesting a role of leptin in more common forms of obesity.

O’Rahilly described another person who was found to have a mutation in the enzyme that processes proopiomelanocortin (POMC) as well as proinsulin and other hormone precursors, suggesting that POMC deficiency may play a role in clinical obesity. Other patients with POMC mutations also show obesity as well as very light skin pigmentation. Animal POMC deletion models exhibit hyperphagia, severe obesity, hyperinsulinemia, and increased linear growth in heterozygotes. Approximately 0.5% of severely obese individuals in O’Rahilly’s program were heterozygous for abnormal POMC. The mechanism of POMC effect on appetite is complex, with hypothalamic arcuate nucleus POMC neurons traced to the spinal cord, suggesting direct peripheral action.

In other studies of ~200 individuals without severe obesity but with severe insulin resistance, >20 missense genetic variations have been detected. O’Rahilly presented findings in a nonobese woman with severe insulin resistance, a history of polycystic ovary syndrome in young adulthood, severe hypertension, a fatty liver, partial peripheral lipodystrophy, and acanthosis nigricans. She had a mutation in the PPAR-γ ligand binding domain, with the same mutation present in her son and grandchildren. This dominant-negative mutation changes the binding of nuclear coactivator molecules to PPAR-γ. Affected individuals have a marked decrease in the adiponectin family adipoocyte hormone Acrp30, which may be related to insulin sensitization, and show clinical improvement as well as increase in Acrp30 with high-dose rosiglitazone treatment. Another mutation in PPAR-γ in an insulin-resistant patient appears to exist in combination with an additional mutation affecting skeletal muscle glycogen synthesis, suggesting an additional aspect of the pathophysiology of diabetes.

Postprandial hyperglycemia

At a symposium on the role of postprandial hyperglycemia in the pathogenesis of diabetic complications, Sudesh Kumar (Birmingham, U.K.) discussed the clinical relevance of postprandial hyperglycemia, reviewing epidemiologic evidence of the close relationship between postload glucose and micro- and macrovascular disease. In the Framingham Study, macrovascular disease increased two- to fourfold. (1) Kumar noted that Scandinavian studies show association of hyperglycemia with cardiovascular disease (CVD) risk for subjects with type 1 diabetes (2). Similarly, the Wisconsin Epidemiologic Study of Diabetic Retinopathy showed a 20% increase in CVD for each 1% rise in HbA1c (3), and in the U.K. Prospective Diabetes Study (UKPDS), there was a relationship between HbA1c and microvascular end points and a 37% decrease per 1% HbA1c, as well as for macrovascular disease, with myocardial infarction de-
increasing 14% per 1% decrease in HbA1c (4).

Most subjects spend the day in the postprandial state, but studies of diabetes focus on HbA1c or fasting glucose, missing the important fluctuations in blood glucose during the day. Postload glucose during glucose tolerance testing may act as a surrogate for postprandial hyperglycemia. A number of studies have shown that nonfasting glucose is more strongly predictive of CVD than fasting glucose in both nondiabetic and diabetic patients. In the Diabetes Epidemiology: Collaborative analysis Of Diagnostic criteria in Europe (DECODE) study, the linkage of hyperglycemia to macrovascular disease was weaker for fasting than for postload glycemias (5). Among 25,364 individuals followed for 7.3 years, within each fasting glucose group, mortality increased with rising 2-h glucose, but within each 2-h glucose group, there was no additional increase in risk with increasing fasting glucose. In the Honolulu Heart Program, glucose measured 1 h after 50-g oral glucose showed a strong linear relationship with CVD death and total CVD at 12- and 23-year follow-up among 8,066 Japanese American subjects (6). In the Diabetes Intervention Study, of 1,139 subjects with diabetes, those with postprandial glucose 3.3–8 mmol/l had lower CVD rates than those with levels >10 mmol/l (7). Comparing fasting and postprandial glucose, triglycerides, and blood pressure, there was no significant effect of fasting glucose on mortality but a clear effect of postprandial glucose as well as of triglycerides and blood pressure.

Possible explanations of the association of postprandial hyperglycemia with adverse outcome include direct toxic effects of glucose via protein kinase C (PKC), increased oxidant stress, or glycation of lipoproteins, or indirect associations, with postprandial glucose elevations perhaps as a manifestation of the metabolic syndrome. Kumar pointed out that individuals with insulin resistance as their major defect have relatively low fasting glucose, whereas those predominantly with a β-cell defect show concordant increase in fasting and postprandial glucose as they progress from normal to impaired glucose tolerance (IGT) to diabetes. In addition, postprandial glucose is associated with postprandial dyslipidemia, higher blood pressure, and a prothrombotic state, all superimposed on the effects of baseline hyperglycemia. Kumar concluded that postprandial glucose is associated with increased risk of macrovascular disease and premature mortality, with fasting glucose not as good a marker, and that because control of postprandial glucose is associated with better HbA1c, we must stop ignoring this as a target in clinical practice.

Antonio Ceriello (Udine, Italy) discussed potential mechanisms of the relationship between postprandial glucose and the pathologic changes of diabetes. He pointed out that the postprandial state lasts for ~18 h per day. In individuals with diabetes, postprandial glucose particularly contributes to mean glycemia, and hyperglycemic spikes may also affect cardiovascular risk factors, perhaps based on oxidative stress due to postprandial changes. After meals, levels of both glucose and of triglycerides increase, and lowering of postprandial glucose is associated with lowering of triglyceride levels (8). There is also correlation between factor VII and postprandial glucose (9). Vasoconstrictive effects of acute hyperglycemia can be reversed by L-arginine, suggesting mediation by decreasing nitric oxide levels (10). Individuals with and without IGT and patients with diabetes show progressive decrease in flow-mediated brachial artery dilation, with inverse correlation between blood flow and the plasma glucose level (11) and oxidative stress as a potential underlying mechanism. Glucose is freely taken up by the endothelium via GLUT1, leading to production of reactive oxygen species such as the superoxide radical (12). The antioxidants glutathione and vitamin C reverse the hemodynamic changes and endothelial dysfunction caused by hyperglycemia, whereas serum antioxidant levels, after an oral glucose load, are lower in subjects with than those without type 2 diabetes (13).

Ceriello noted that toxic oxidation products such as lipid peroxides and advanced glycation end products (AGEs) are produced by cooking, frying, and storing food. In the gastrointestinal tract, these products are affected by pH and by catalytic metals and enzymes, potentially further increasing oxidative stress. Thus, food choices may be of great importance, both for foods low in these products and food antioxidants. In diabetes, the simultaneous presence of hyperglycemia may further contribute to oxidative stress and decrease detoxification of food oxidation products. Increased malondialdehyde (MDA) levels and decreased total plasma radical-trapping parameter (TRAP) are seen in individuals with diabetes during meals (14), and high carbohydrate meals increase glycemia, with greater fall in TRAP and greater increase in MDA, accompanied by an increase in LDL oxidation (15).

Stefano del Prato (Pisa, Italy) compared fasting glucose and HbA1c in the UKPDS. Both had increased by 5% at 3 years and by 10% at 5 years, but fasting glucose increased 12 and 15% and HbA1c increased 15 and 20% at 7 and 9 years, respectively, suggesting that treatment based on fasting glucose alone fails to maintain similar control of glucose excursions. This is further suggested by studies showing stronger correlation between postprandial glucose and HbA1c than those between fasting glucose and HbA1c. In the DCCT (Diabetes Control and Complications Trial), the intensive treatment group had less retinopathy than the conventional group, even after controlling for HbA1c. A possible explanation for this is greater control of glucose excursions with intensive treatment (16). Del Prato pointed out that 24-h continuous subcutaneous glucose monitoring shows considerable difference between individuals in glycemic variability for a given level of HbA1c, suggesting that the measurement of amplitude of glycemic excursions, originally proposed >20 years ago, may be an important tool for assessing the diabetic state (17).

In addition to the usual measurement of stable glycohemoglobin, other analyses may be useful for assessing glycemic variability. Del Prato showed a stronger correlation of labile than stable glycohemoglobin with recent glucose levels, with the labile glycohemoglobin also correlating with the SD and coefficient of variation of plasma glucose on the day prior to measurement. Comparing type 1 diabetic with type 2 diabetic patients, the former have greater glucose variability, and fructosamine measurement appears to be more useful in demonstrating this than HbA1c measurement (18). Postprandial glucose levels >180 mg/dl appear to be associated with increasing retinopathy and nephropathy (19). Del Prato noted that 2-h postload glucose shows a stronger correlation with carotid intima-media thickness (IMT) than HbA1c. There is also
a relationship between postprandial triglyceride and carotid IMT, with insulin, C-peptide, and total, HDL, and LDL cholesterol not significant in multivariate analysis controlling for postload glucose and triglyceride levels (20). Plasma 1.5 anhydroglucitol shows an inverse relationship with postprandial glucose, and may be useful as an indirect measure of this parameter, showing a greater correlation with glucose variability than HbA1c (21). Another potential measure is of ketohydrates or dicarbanyls such as methylglyoxal, which may increase oxidative stress and are directly related to postprandial glucose excursions (22). These compounds may be excessively short-lived to be useful in clinical assays, and glyctated dicarbanyls may prove better markers. Lower levels of 3-deoxyglucosone and 3-deoxyfructose are seen in individuals with lower glucose variability at similar HbA1c levels (23).

Thomas Pieber (Graz, Austria) ended the symposium on a contrary note, suggesting a different approach to the question of whether there is evidence to treat postprandial hyperglycemia. He cautioned that this may not truly be a modifiable cardiovascular risk factor and recalled a statement Brecht attributed to his fictionalized Galileo in a play: “The chief cause of poverty in science is imaginary wealth. The chief aim of science is not to open a door to infinite wisdom but to limit infinite error.” Pieber pointed out that although there are no intervention studies yet available, in the DECODE study, 613 patients had impaired fasting glucose (IFG) only, 431 had both IFG and IGT, and 473 had IGT only, suggesting mainly postload glycemia. IFG was associated with considerably lower CVD mortality than IGT, for which mortality was identical to that of frank diabetes (24). Similar findings were reported in a summary of outcome among 9,297 participants in three prospective studies in the South Pacific, with isolated fasting hyperglycemia having lower risk than postprandial hyperglycemia (25). Other studies, however, suggest association of cardiovascular risk with fasting glucose. Analysis of 20 prospective studies of 95,783 nondiabetic subjects during 12.4-year follow-up showed risk with increasing fasting and 2-h glucose (26). Interestingly, the two glycemic measures are less strongly related in very obese individuals. Of further importance is the relationship between HbA1c and CVD in nondiabetic populations. A 2- to 4-year follow-up of 4,662 men showed progressive increase in CVD risk, as HbA1c increased from 5–5.4 to 5.4–6.9 to >7% to known diabetes (27). Multivariate analysis of this data showed a 1.5-fold increase in relative risk for each 1% increase in HbA1c, regardless of whether individuals with diabetes were included. Thus, fasting and postprandial glucose and HbA1c are all continuous risk factors in the normal population.

More germane, Pieber stated, is the question of whether glucose is a modifiable risk factor in diabetes. “We have to tackle the proven risk factors,” he stated, and he questioned whether postprandial glucose treatment will prove a useful measure in this sense. Pieber noted the 12% reduction in total diabetes-related end points in the UKPDS, which he characterized as being “somehow a little bit disappointing,” particularly because there was no significant effect on diabetes-related mortality. Based on the UKPDS data, one would need to treat 20 patients to prevent one diabetes-related end point, 36 patients to prevent and one microvascular complication, and 100 patients to avoid one death (based on the nonsignificant change in mortality). Pieber concluded that the effect of glycemia on risk is weak and that an evidence base allowing one to further distinguish between postprandial and fasting glycemia does not exist.

**Primary prevention of type 2 diabetes**

Karl-Fredrick Eriksson (Malmo, Sweden) noted the association between diabetes and adverse outcome and the high frequency of complications at the time of diagnosis. Studies from his center show that men with family history of both diabetes and hypertension have a 30% increase in CVD risk and that positive family history of diabetes and evidence of the insulin-resistance syndrome is associated with a threefold increase in risk. IGT precedes diabetes by years to decades. In principle, treatment at the time of recognition of IGT could decrease adverse outcome. This hypothesis has now been addressed in a number of controlled studies.

In a study in Malmohus, Sweden, 14% of those treated with diet versus 29% with no treatment developed diabetes over 12 years, and none of those treated with tolbutamide developed diabetes, with the latter group showing a decrease in CVD and total mortality, although because of small numbers, this was not significant (28). In a study in Malmo, 288 men aged 40 years participated in an active intervention group with diet, exercise, or both. Those treated with diet had 7% weight loss, and physical exercise improved maximal oxygen uptake. Both groups improved glucose tolerance. The benefits on weight and fitness were maintained at 6 years. Eleven percent in the intervention group versus 29% of those not receiving diet or exercise instruction developed diabetes (29). At 12-year follow-up, mortality was 6 vs. 15% in patients with IGT not receiving treatment. This pattern continued at 19-year follow-up, with the intervention group showing similar mortality to that of the normal (non-IGT) population. In multivariate analysis, the intervention was predictive of mortality, controlling for BMI, cholesterol, blood pressure, and cigarette use. In the Da Qing study, 68% of control patients developed diabetes over 6-year follow-up, whereas with diet, exercise, or both, 41–46% developed diabetes (30). Eriksson emphasized the importance of physical activity and diet as potential interventions.

Jaakko Tuomilehto (Helsinki, Finland) discussed the Diabetes Prevention Study, in which lifestyle advice was given to 522 individuals with IGT based on two glucose tolerance tests, with a 35% estimated 6-year risk of diabetes. Mean age was 55 years, BMI 31 kg/m², and waist circumference averaged 102 cm. Of the intervention and control groups, 91 and 81%, respectively, were women. Diets were high in saturated fat and low in fiber. The intervention group was given five targets: a 5% weight loss, <30% total and <10% saturated fat intake, >15g/1,000 kcal fiber, and aerobic plus muscle strengthening exercise for at least 30 min daily. Individual diet and exercise counseling was provided seven times during the first year and four times annually thereafter. The control group was given “general advice about healthy diet and exercise” without individual counseling. Of the intervention and control groups, 91 and 93%, respectively, participated throughout the study.

Self-reported change in fat intake and exercise were seen in 87 and 30% vs. 70 and 16% of the intervention versus control groups, with food records suggesting
a decrease in energy intake of 250 vs. 106 kcal. Weight decreased 4.2 and 3.5 kg at 1 and 2 years in the intervention group while decreasing 0.8 kg at both 1 and 2 years in the control group. Plasma triglyceride decreased 17 mg/dl with the intervention versus 1 mg/dl in the control group. There was strong correlation between the changes in BMI, fat mass, and waist and hip circumference. At 6 years, diabetes had developed in 20 and 40% in the control and intervention groups, respectively, with a 58% decrease in diabetes in the intervention group. For control versus intervention, the weight reduction target was achieved in 43 vs. 13%, total fat targets in 47 vs. 26%, saturated fat targets in 26 vs. 11%, and 86 vs. 71% exercised >4 h/week. Those intervention group members who achieved no lifestyle targets showed a 30–35% rate of development of diabetes, whereas none of those who achieved either four or all five targets developed diabetes. Those with weight loss >5% decreased diabetes by 74 vs. 60% in the intervention versus control group, with every 3-kg weight loss doubling the benefit, and those ingesting >15 g/1,000 kcal fiber decreased diabetes by 77 vs. 60%. All five targets showed high correlation with each other. Tuomilehto concluded that lifestyle intervention is feasible and beneficial and decreases the incidence of type 2 diabetes (31,32).

David Marrero (Indianopolis, IN) described the design of the Diabetes Prevention Program (DPP). He discussed the long period of glucose intolerance that precedes diabetes. Obesity, body fat distribution, physical inactivity, and elevated fasting and 2-h glucose were all assessed in the study. A total of 27 centers followed a common protocol, with 1,079, 1,073, and 1,082 individuals >24 years of age with BMI at least 24 kg/m² and with IGT based on 2-h glucose 140–199 mg/dl and fasting glucose 95–125 mg/dl randomized to intensive lifestyle intervention, metformin, or placebo groups, respectively. A total of 158,177 individuals were screened, 30,985 had an oral glucose tolerance test, and 4,719 were enrolled in a run-in phase to assure compliance. Of those enrolled, 68% were women. The lifestyle intervention encouraged loss of at least 7% of initial body weight, primarily by decreasing fat to 25% of total calories and calories to 1,200–1,800 kcal per day, and at least 150 min of activity weekly similar to brisk walking was encouraged in a 16-session core curriculum over 24 weeks. A case manager contacted each patient at least monthly by telephone and at least every 2 months in person, and access to dieticians and exercise physiologists was readily available, with supervised exercise sessions. Metformin was administered at a dose of 850 mg once daily for 1 month and twice daily thereafter, with a decrease to one-half tablet if gastrointestinal intolerance occurred.

William C. Knowler (Phoenix, Arizona) presented the findings of the DPP. There was an average of 2.8 years of follow-up. Of patients in the lifestyle group, 74% performed at least 150 min of activity weekly, with weight loss of 7 kg compared with 3 and 0 kg in the metformin and placebo groups, respectively, at 1 year. A 4-kg weight loss was maintained in the lifestyle group at study completion. For the lifestyle, metformin, and placebo groups, diabetes developed cumulatively at 3 years in 14, 22, and 29%, respectively, a reduction of 58 and 31% in the lifestyle and metformin groups, respectively, with both interventions statistically different from each other and placebo. Treatment of somewhat fewer than seven subjects with the lifestyle intervention would prevent one case of diabetes at a cost of $15,000. The lifestyle intervention was more effective and metformin treatment less effective in older (>60 years of age) individuals. Those with BMI 24–30 kg/m² showed no benefit of metformin, whereas lifestyle and metformin were similarly effective in those with BMI >36 kg/m². The metformin effect was greater in individuals with higher baseline fasting glucose. Normal glucose tolerance was seen in more participants with lifestyle intervention than with metformin treatment or placebo. Metformin was associated with a 78% frequency of gastrointestinal complaints.

T. Buchanan (Los Angeles, CA) discussed the TRIPOD (Troglitazona In the Prevention Of Diabetes) study, in which Hispanic women with gestational diabetes and a 70% 5-year risk of diabetes were treated with either 400 mg troglitazone (TGZ) daily or placebo. Totals of 121 placebo and 114 TGZ-treated women aged 35 years with BMI 31 kg/m², waist-to-hip ratio 0.86, and two-thirds with IGT were studied for 30 months, with diabetes developing in 12.3 vs. 5.4% annually. Assessing which early metabolic changes were associated with prevention of diabetes, intravenous glucose tolerance testing showed improved insulin sensitivity and decreased insulin secretion with TGZ at 3 months. Dividing participants into tertiles of improvement in insulin sensitivity and decreases in insulin secretion, those with the smallest change in sensitivity had no protection, and the greatest protective factor was the decrease in insulin secretion with TGZ treatment, suggesting that reducing the workload for the pancreas was of greatest importance. There was no baseline difference between responders and nonresponders, with responders having a 9.8% and nonresponders having a 3% rate of development of diabetes annually. Those in the tertile of greatest reduction in insulin secretion with TGZ treatment were completely protected against the development of diabetes. At 8 months after study completion, 39 and 44 of TGZ and placebo patients, respectively, were reassessed, with 3 vs. 18% annual diabetes rates and with stable versus deteriorated insulin secretion-sensitivity ratios, suggesting true protection. Thus, TGZ decreased type 2 diabetes by 56%, with protection extending to 8 months after drug withdrawal, suggesting that reducing secretory demands placed on β-cells by chronic insulin resistance can delay or prevent the onset of type 2 diabetes. When asked whether administering insulin to produce complete β-cell rest might also be effective, Buchanan agreed, although he suggested that this might be impractical.

Jean-Louis Chiasson presented the results of the STOP-NIDDM (Study to Prevent NIDDM) trial carried out in Canada, Germany, Israel, Spain, and the Scandinavian countries to evaluate the effect of acarbose treatment on the conversion rate of IGT to type 2 diabetes. Glucose tolerance, insulin secretion and sensitivity, lipids, blood pressure, CVD, and anthropometric changes were followed (but not presented at this session). Patients aged 40–70 years with fasting blood glucose >5.6 mmol/l and IGT were recruited. Approximately 10,000 glucose tolerance tests were performed. Of screened subjects, 21.6% had type 2 diabetes and 17.5% had IGT, with 12.2% having fasting glucose >5.6 mmol/l. A total of 1,429 subjects were enrolled in the study, with 715 randomized to placebo and 714 to 100 mg acarbose t.i.d. After at least 3 years of treatment, a 3-month pla-
A carbo washout period was given, with mean study duration of 3.6 years. A total of 17 subjects were excluded early in the study because of the presence of diabetes, and 44 were excluded because of nonparticipation, leaving an intent-to-treat population of 1,368 subjects balanced between acarbose and placebo. The mean BMI was 31 kg/m², >70% had dyslipidemia, and the fasting and 2-h glucose were 112 and 166 mg/dl, respectively. There were more dropouts with active treatment, 131 vs. 34 patients, with >60% discontinuing during the first year. Of those who withdrew, 55% of those who had been randomized to acarbose and 27% randomized to placebo developed diabetes. A total of 35.3% reverted to normal glucose tolerance, 28.4% continued to have IGT, and 32.8% developed diabetes with acarbose, whereas 41.8% developed diabetes with placebo. Cumulative diabetes rates were therefore 9% lower with acarbose, for a relative risk reduction of 21%, giving a risk ratio of 0.76 to time of onset of diabetes; therefore, 11 individuals would have to be treated for 3 years to prevent one case of diabetes at a cost of $7,800. Body weight decreased 1 vs. 0 kg in the acarbose versus placebo groups. Three months after treatment, diabetes rates were 39.7 vs. 44.3%, suggesting fairly rapid loss of protection against diabetes after discontinuing treatment. Ciaras noted the relatively high noncompliance rate, and pointed out that those who adhered to treatment had greater protection.

The immune system and atherosclerosis

Dor Harats (Tel Hashomer, Israel) gave a state-of-the-art lecture on the role of the immune system in atherosclerosis, addressing the relationship between inflammation and atherosclerosis, which has recently gained prominence with the recognition that statin treatment may have specific anti-inflammatory actions independent of their lipid-lowering effects (33). Thus, in addition to the traditional risk factors of dyslipidemia, diabetes, hypertension, and smoking, a number of biological mediators of inflammation, including T-cells and macrophages, appear to be crucial to the development of the atherosclerotic lesion, causing endothelial dysfunction and the hypercoagulable state. Atherosclerosis, Harats pointed out, starts with foam cells entering the blood vessel early in life, and “the immune system cannot stay indifferent to it,” particularly in mediating the development of acute events that occur at sites of arterial wall inflammation. Repair of the atherosclerotic lesion may therefore require agents to decrease inflammation, either indirectly by lowering lipid or oxidized lipid levels or by direct anti-inflammatory actions.

Antigen-presenting cells and T-cells are present in atherosclerotic plaques.

Evidence that an antigen is involved includes its presence in the lesion, the triggering of humoral and cellular responses, immunization with the antigen affecting atherosclerosis, transferrable immunity, and decreased disease when the immune response is suppressed. A potential autoantibody is oxidized LDL (oxLDL). Both antibodies and T-cell clones recognizing oxLDL are present in atherothrombotic plaques, and there is evidence that oxLDL drives T-cell responses. Immunization of mice not expressing apoE with oxLDL leads to T-cell–dependent antibody formation, which is protective against atherosclerosis (34). Another antigenic protein may be heat shock protein (HSP)-65. Antibodies to HSP-65 are associated with carotid atherosclerosis and mediate endothelial dysfunction, whereas immunization with HSP-65 increases atherosclerosis, which can then be reduced by administration of an anti-CD3 antibody and prednisone to block T-cell function. An adoptive transfer study showed that administering HSP-65–reactive T-cells to mice lacking LDL receptor increased atherosclerosis. Another antigen, β2-glycoprotein (GP)-1, is an in vivo anticoagulant that probably mediates the anti-cardiolipin syndrome. Anti β2-GP-1 is associated with premature atherosclerosis, and these antibodies activate endothelial cells, with increased expression of adhesion molecules as well as increased oxLDL uptake. Immunization with human anti-β2-GP-1 IgG from a patient with anti-cardiolipin syndrome induced atherosclerosis in LDL receptor null mice (35). Further studies have shown that human atherosclerotic lesions are positive in immunohistochemical staining for this antibody, which localizes with CD4 and CD8 T-cells. The antigen is incorporated into macrophages in atherosclerotic lesions as well, and adoptive transfer of β2-GP-1–reactive T-cells can cause atherosclerosis. Thus, HSP-65 and β2-GP-1 may be protective, with immune response to them worsening atherosclerosis, whereas oxLDL is harmful, with immune response improving atherosclerosis.

Activated T-cells precede macrophages in the development of the atherosclerotic plaque.

Cytokines and chemokines, including interleukin (IL)-2, IFN-γ, and tumor necrosis factor-α are secreted by these cells. Absence of mature T- and B-cells impedes development of early atherosclerosis in rodent models not expressing either apoE or the LDL receptor. There are three divergent potential T-cell responses, Th-helper (Th)1, Th2, and Th3. Th1-producing IFN-γ is present in atherosclerosis, with IFN-γ influencing the scavenger receptor. Statins appear to act as immunomodulators, decreasing IFN-γ–induced antigen presentation and cytokine formation. Pentoxifylline inhibits the Th1 differentiation pathway and may have antiatherosclerotic effects. The Th2 differentiation pathway is protective, leading to increased IL-10, which decreases IFN-γ and decreases atherosclerosis. IL-4 is another anti-inflammatory cytokine produced by Th2 cells and is involved in HSP-65 immune-mediated fatty streak formation. Transforming growth factor (TGF)-β is also protective and is expressed in atherosclerotic lesions by Th3 T-cells.

Harats noted that some T-cell activation occurs without antigens as an effect of IL-15, which is expressed in atherosclerotic lesions. Innate immunity is another aspect of the immune portion of atherosclerosis. The innate immune system plays a crucial role in the acute response to infectious agents, clearing microorganisms within hours of exposure. Subsets of lymphocytes that recognize pathogenic determinants are expressed even in immunologically naive animals, leading to strong protective responses to viral and bacterial infections—an “evolutionary memory.” There appears to also be innate immunity to oxLDL (36). The consequent production of T15 IgM antibodies to oxLDL may have a protective effect against atherosclerosis.

Immunosuppression might be considered as a potential approach to treatment, but this actually appears to enhance atherosclerosis, perhaps because of suppression of protective immune factors. Immunoglobulin treatment decreases
atherosclerotic lesions in apoE null mice. When anti-CD 40 is administered, there is a decrease in aortic atherosclerosis. Another potential treatment would involve oral administration of antigen, which, in low dose induces Th2 and Th3 response, which can actively suppress atherosclerosis, and which in high dose may block the Th1 response, further decreasing the atherosclerotic response. In both LDL receptor and apoE null mice, administration of oxLDL or an oxidized phospholipid analog orally decreased atherosclerotic lesion size and decreased T-cell response. Oral HSP-65 administration also decreases atherosclerosis, and oral β-2GP-1 induces Th2 cytokine production and appears to decrease atherosclerosis progression.

A model of increased atherosclerosis is the streptozotocin (STZ)-diabetic mouse not expressing the LDL receptor. Administration of insulin to these animals decreases lesion formation, although it does not lower cholesterol levels. HSP-65 immunity in diabetic LDL receptor null mice also decreases atherosclerosis. STZ-diabetic mice show low IL-10 and high IFN-γ, suggesting a shift from Th2 to Th1 lymphocytes, potentially increasing the atherosclerotic process. Thus, we need to understand a great deal more about the biology of atherosclerosis to develop effective treatment strategies.

Potential therapeutic strategies for diabetic nephropathy

At the 16th Camillo Golgi lecture on potential therapeutic strategies for diabetic nephropathy, Allan Flyvbjerg (Aarhus, Denmark) discussed nephropathy, which affects 25–30% of type 1 diabetic subjects and 15–20% of type 2 diabetic subjects. In type 1 diabetes, nephropathy begins with increased kidney volume and hyperfiltration, followed by glomerular changes, microalbuminuria, macroalbuminuria, and finally the development of end-stage renal disease. The pathogenesis involves effects of glycemia, blood pressure, environmental influences such as cigarettes and diet, and a number of unidentified or not well-characterized factors, including growth, metabolic, and vasoactive factors. Elucidation of the latter requires careful choice of animal models, with the best rodent type 1 diabetes models including STZ and alloxan and the autoimmune spontaneously diabetic BB rat and NOD mouse. Within weeks, these animals show extreme renal growth and hyperfiltration, with matrix accumulation occurring for several months. Flyvbjerg discussed growth factors, such as growth hormone (GH), IGF-1, and TGF-β, and nonglucose metabolic factors, particularly PKC and AGEs, playing a role in these processes.

The study of the relationship between diabetes and GH began with studies by Bernardo Houssay in 1936 that showed the diabetogenic effect of GH, and Prange-Hansen in the 1970s showed increased GH in patients with diabetes, with evidence of its relationship to diabetic retinopathy. It was subsequently recognized that in diabetes, however, hypoinsulinemia and poor metabolic control cause resistance to action of GH, decreasing IGF-1 and IGF-binding protein (BP)3 and increasing IGF-BP1, resulting in a decrease in free IGF-1 levels. In diabetic dwarf rats with GH deficiency, there is a marked decrease in the development of albuminuria. Further evidence of protection against nephropathy is seen in a diabetic animal model lacking the growth hormone receptor (37). Diabetic animals treated with the long-acting somatostatin analog octreotide show similar effects as in diabetic dwarf rats. The development of GH and IGF-1 receptor antagonists has further shown the role of GH in diabetic nephropathy, with experimental treatment with GH antagonists decreasing albuminuria and glomerular hypertrophy. PKC activity and levels of diacylglycerol (DAG) are elevated in numerous tissues prone to diabetic complications. There are 11 PKC isoforms, PKC-β1 binds DAG, PKC-ε is DAG sensitive, and PKC-θ is DAG insensitive. Specific PKC-β inhibitors and perhaps antagonists of the receptor for activated C kinase anchoring proteins may become useful in the future. PKC-β inhibitors do not decrease kidney size but markedly decrease glomerular volume and albuminuria without effect on nondiabetic control subjects. The inhibitors also decrease TGF-β and fibronectin levels in some studies as well as reduce mesangial expansion in type 2 diabetes models.

**AGE and its four receptors are involved in diabetic nephropathy.** AGE binds to the receptor for AGE (RAGE), leading to signal transduction involving nerve factor (NF)-κB. Diabetic transgenic mice overexpressing RAGE show acceleration of nephropathy, with increased kidney size, increased albuminuria, and increased mesangial fraction (38). AGE formation inhibitors, AGE cross-link inhibitors, soluble RAGE, and neutralizing RAGE antibodies may theoretically decrease the development of diabetes-related renal changes. A novel AGE formation inhibitor, ALT-946, decreases albuminuria and glomerular filtration rate in STZ rats (39). This may prove safer than the previously studied inhibitor of AGE formation, amidoguanidine. Using a specific RAGE-blocking antibody in a type 2 diabetes model, decreases have been shown in kidney weight, albuminuria, glomerular volume, and creatinine levels. Studies of AGE cross-link inhibitors and soluble RAGE have not been performed but will be of interest.

Flyvbjerg concluded that therapeutic efforts to address the growth factor, cytokine, PKC, AGE, and vasoactive factors mediating diabetic nephropathy will all be of importance and noted that many aspects of these systems overlap, suggesting a common underlying pathogenesis by these differing mechanisms that has not been fully elucidated.

**References**


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