# Glycation Inactivation of hCD59 May Lead to Diabetic Complications

The cellular and molecular mechanisms for micro- and macrovascular disease are incompletely understood.

**REVIEWED BY JOSE A. HALPERIN, MD**

Extensive epidemiological evidence has demonstrated that prolonged hyperglycemia underlies the chronic complications seen among diabetic patients. Treatment of these complications accounts for close to 15% of health care expenditures in the United States.

Researchers reporting in *Diabetes* said that hyperglycemia drives protein glycation; possibly leading to the formation of advanced glycation end products (AGEs). "When glycation involves its active site, the glycated protein may lose function, as reported for glycated hemoglobin or AGE basic fibroblast growth factor," wrote Jose A. Halperin, MD, and colleagues.

## CONSISTENT CORRELATION

A strong correlation found among hyperglycemia, protein glycation and AGE formation is consistent with a role of protein glycation in the pathogenesis of diabetic complications, they wrote. While several mechanisms have been reported as contributing to hyperglycemia-related pathology, the cellular and molecular basis for vascular damage is not completely known.

Study authors proposed that inactivation by glycation of the complement regulatory protein human CD59 (hCD59) is a novel mechanism that contributes to the increased vascular risk in people with diabetes. They explained that the complement system is made up of more than 12 soluble proteins that interact in three enzymatic cascades. These converge to form membrane attack complex (MAC).

MAC is a polymer that is circular in shape and made up of 12 to 18 monomers of the C9 complement protein. MACs can insert themselves into cell membranes and form a transmembrane pore. Salt and water travel through the pore and cause swelling and lysis of MAC-targeted cells.

> More than 10 evolved inhibitory proteins restrict the complement activation in the activation pathways.

"We have demonstrated that during the assembly of the MAC pore, there is a phase when the pore is transient and reversible, permitting opening and closing/resealing of the plasma membrane," they wrote.

Dr. Halperin and colleagues said that transient MAC pores generate changes and mediate physiological and/or pathological responses. "For example, complement activation and nonlytic MAC formation on endothelium activates endothelial cells and induces the release into the extracellular medium of growth factors and cytokines," they wrote. These molecules do three things: 1) promote proliferation of fibroblasts as well as endothelial and smooth muscle cells, 2) promote inflammation and attract monocytes and macrophages to the site of activation, thus inducing the expression of proinflammatory adhesion molecules, and 3) promote thrombosis by inducing the expression of tissue factor and the exposure of binding sites for clotting factor Va.

## DOWN REGULATE

The researchers explained that in order to down-regulate complement activity and protect "self" cells from these catastrophic effects, more than 10 inhibitory proteins have evolved. They restrict the complement activation at

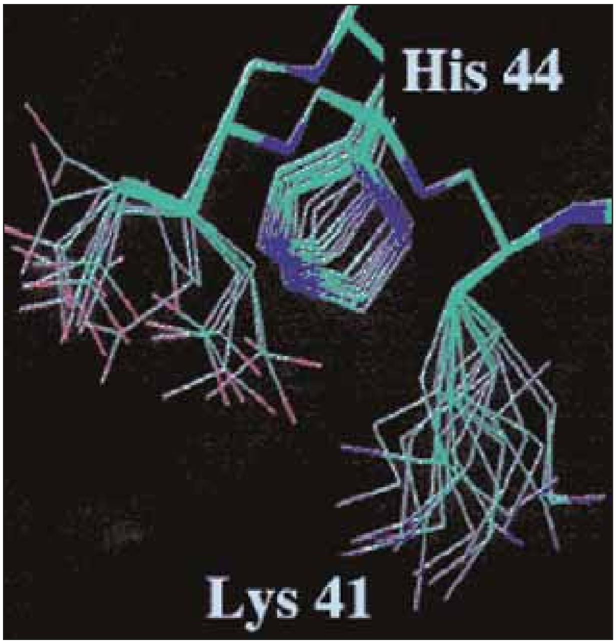


**Figure 1. The nuclear magnetic resonance structure of CD59 shows a lysine close to a histidine, a motif that marks the complement regulator as a potential glycation target.**

different stages in the activation pathways. CD59 is one of these proteins; it specifically inhibits formation of the MAC.

On the basis of previous research,[2] the investigators proposed that glycation inactivation of hCD59 in diabetes could increase MAC deposition in diabetic tissues and link the complement system to the pathogenesis of diabetic vascular complications. "The previously reported findings of increased MAC deposition in diabetic kidneys, nerves and retinas are consistent with this hypothesis, but the mechanism that induces MAC deposition in diabetic tissues has not been investigated in humans," they wrote.

## FINICKY REACTION

Glycation is a finicky reaction that only targets certain amino acids, preferring a lysine with a histidine nearby. Halperin's group found this lysine-histidine duo in the structure of human CD59 – lysine 41 and histidine 44 – lie within the distance characteristic of a glycation site. Furthermore, the lysine is adjacent to tryptophan 40, the only amino acid that is known to be essential for CD59's function. Glycation of this site cripples CD59 function. One of the most intriguing implications of this finding is that it may explain the unique propensity of humans to develop vascular complications in the combination and with the intensity seem in human diabetes. As it turns out, humans are the only known species to have the lysine-histidine pair next to the active site of CD59 (Figure 1), making the inhibition of CD59 through glycation a human phenomenon.

Dr. Halperin and colleagues reported on a immunocytochemistry study in diabetic and nondiabetic kidney and nerve biopsies in order to show the presence of glycated hCD59 colocalized with MAC in the diabetic but not the nondiabetic tissues (Figure 2). "We also show that erythrocytes of diabetic patients have a significantly reduced activity of hCD59 and increased sensitivity to MAC-mediated lysis, as expected from inactivation of CD59," they wrote.

## EXPLANATION FOR MAC DEPOSITS

Taken together, the investigators believe that findings indicate that glycation inactivation of the complement regulatory protein hCD59 may explain the increased MAC deposits in diabetic tissue and contribute – at the molecular level – to vascular proliferative pathology that creates diabetic complications.

Among the results of the immunocytochemistry study, Dr. Halperin and colleagues reported:

- Extensive MAC deposition in a kidney that is transplanted into a diabetic patient to treat diabetic nephropathy, which had no histological evidence of late rejection.
- The generation of an antibody that specifically recognizes glycated but not nonglycated hCD59.
- MAC and glycated hCD59 colocalized in diabetic human kidneys and nerves (Figure 2).
- Decreased hCD59 activity in diabetic red blood cells, which consistently exhibit an increased sensitivity to complement-mediated lysis.

"The results suggest that increased MAC-induced signaling as a result of glycation inactivation of hCD59 could act synergistically with other hyperglycemia-related pathways to produce the long-term vascular complications of human diabetes," the investigators concluded.

In an interview, Dr. Halperin said that it is important for both physicians and scientists to understand the pathophysiology of diseases and their complications at the molecular level. This is an essential step to design interventions to prevent disease or sequelae.

"Understanding a new molecular pathway provides the opportunity to develop treatments to affect these pathways in the future. The relevance of CD59 glycation-inactivation to the development of diabetic vascular disease is clear: Inactivated CD59 allows for more MAC deposition, which in turn induces the release of growth factors and cytokines that promote proliferation, inflammation and thrombosis," Dr. Halperin said. "The challenge that lays before us is to translate this novel pathogenic mechanism into tools that

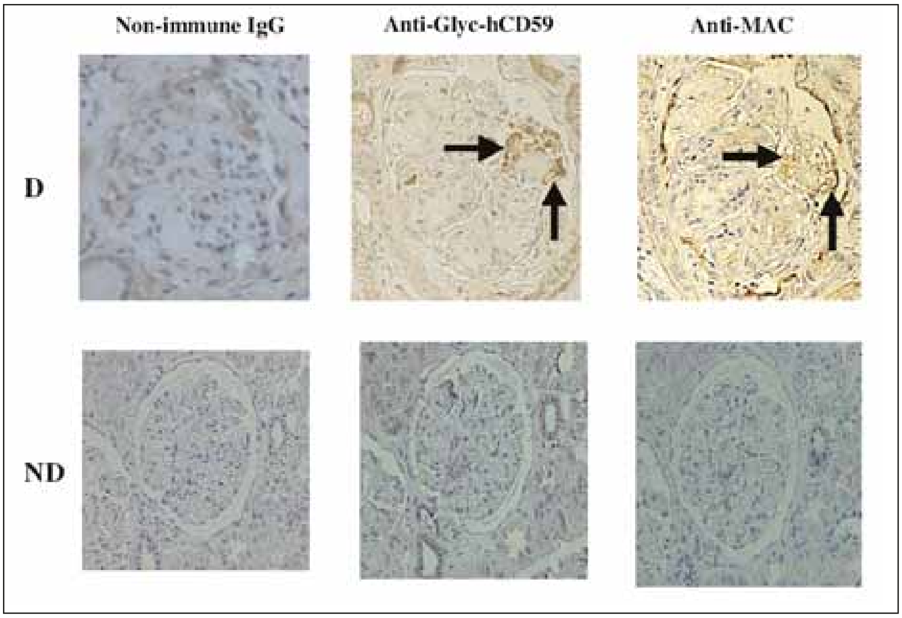


**Figure 2. Glycated hCD59 colocalizes with MAC in diabetic human kidneys. The arrows indicate positive staining areas for glycated CD59 and MAC in serial sections of the same glomerulus from a diabetic individual. No staining was found in nondiabetic kidneys.**

would help clinicians influence and/or prevent the development of diabetic complications."

One clinically relevant tool would be a test that allows the detection and quantitation of glycated CD59 in fluids such as urine and plasma, Dr. Halperin said.

## POTENTIAL NEW THERAPIES

In addition to the general development of better alternatives to prevent protein glycation by improving glycemic control, the potential for new therapies based on this data include:

- The development of methods to alter the ability of the sugars to interact with proteins in general, or with the CD59 protein in particular. In this regard, the identification by the same research team of the glycation motif in human CD59 may help engineer molecules that may specifically block glycation of human CD59.
- Development of drugs that alter complement activity in general and MAC formation in particular may be useful in modulating the vascular consequences of hyperglycemia.

"One very important question opened by these studies is whether glycation of CD59 plays a role in the two- to three-fold increased cardiovascular risk documented in individuals with impaired glucose tolerance [IGT] but without overt diabetes," Dr. Halperin said. "The already large and progressively increasing population with IGT make the identification of new, easy and reliable tests for IGT a major public health priority."

Another important question is whether levels of complement activity, known to vary among individuals, influence the risk of complications of diabetes including nephropathy, cardiovascular disease and even bypass graft survival, Dr. Halperin said. "A corollary of this question is whether different levels of complement activity account for or contribute to the excess risk of diabetic complications observed in minority populations."

At present, the biggest problem for clinicians in regard to managing patients with diabetes is get patients to "buy-in" on the necessary lifestyle changes necessary to improve their health, Dr. Halperin said.

"These begin with healthy eating habits and exercise that are (sadly) poorly adhered to by the patients who need them most. Multiple medications are available, but patients do not like the polypharmacy required for maximum risk reduction," he said.

"New treatments are likely to add to the current regimens, not replace them. Patients usually want magical cures. It is hard to convince them that they must participate in the process and that they must be active in their own self care," Dr. Halperin said. "In this regard, a clinical test that measures a pathogenically relevant factor or protein that can be closely monitored and shown to patients as it moves up and down with their compliance to treatment and lifestyle changes indicated by their physicians, may help clinicians in the daily fight against one of the most prevalent, serious and costly human diseases," he said. ■

*Jose A. Halperin, MD, is from the hematology division, department of medicine, Brigham and Women's Hospital and Laboratory for Translational Research, Harvard University. He can be reached at jose_halperin@hms.harvard.edu.*

1. Qin X, Goldfine A, Krumrei N, et al. Glycation inactivation of the complement regulatory protein CD59. A possible role in the pathogenesis of the vascular complications of human diabetes. *Diabetes.* 2004;53:2653-2661.
2. Acosta J, Hettinga J, Fluckiger R, et al. Molecular basis for a link between complement and the vascular complications of diabetes. *Proc Natl Acad Sci U S A.* 1997;10:5450-5455.