Deciphering the complexities of human β cell physiology is critical to our understanding of the pathophysiology behind both type 1 and type 2 diabetes. One way to do this is to study individuals with congenital hyperinsulinism (CHI), a rare genetic disease characterized by dysregulation of insulin secretion resulting in hypoglycemia. In this issue of the JCI, Henquin et al. report in vitro studies of pancreatic tissue obtained from CHI patients during therapeutic pancreatectomy that have yielded exciting new insights into human β cell physiology. The data validate and extend observations made in model organisms.

β Cell dysfunction lies at the center of both major forms of diabetes, the incidence of which is increasing at an alarming rate in both the developed and the developing world (1). In type 1 diabetes mellitus (T1DM), autoimmune β cell destruction results in complete insulin deficiency, whereas in type 2 diabetes mellitus (T2DM), subtle defects in β cell functional mass result in progressive disease. Studies using animal models and cell lines from various sources have yielded invaluable information that has allowed us to better understand the processes responsible for β cell function, dysfunction, replication, and survival. However, since the model systems may not always accurately reflect in vivo human physiology, validation studies in humans are crucial. The crunch comes, however, in that performing such studies is at best difficult and often impossible. Genetic manipulation in vivo, a hugely powerful tool in animal-based research, is obviously impossible in humans, and in vitro genetic manipulations of primary human tissues are difficult and in themselves fraught with pitfalls.

One way to overcome some of these problems is to identify and study individuals with naturally occurring genetic mutations. These cases are rare and often difficult to study, and the results may be difficult to interpret. With animal models, experiments can be performed in genetically identical animals under controlled conditions. In human studies, however, each patient typi-
commentaries

Figure 1
Schematic representation of the major genetic etiologies of CHI. Some of the major pathways regulating insulin secretion are shown. The proteins encoded by genes mutated in different forms of CHI are shown in red. Mutations in either ABCC8 (which encodes SUR1) or KCNJ11 (which encodes Kir6.2) result in defective K\textsubscript{ATP} channel activity and continuous membrane depolarization regardless of glucose levels. Activating mutations in glucokinase (GCK) result in increased glucose metabolism at low circulating glucose levels, resulting in a decreased threshold for glucose-stimulated insulin secretion and thus inappropriate insulin secretion in the presence of hypoglycemia (18). Glutamate dehydrogenase 1 (GLUD1) mutations result in increased conversion of glutamate to \(\alpha\)-ketoglutarate (\(\alpha\)KG), thereby increasing ATP production and insulin secretion (19). Solute carrier family 16, member 1 (SLC16A1) promoter mutations result in inappropriate expression of this transporter in \(\beta\) cells, allowing pyruvate and lactate to enter the cell. Pyruvate can then enter the Krebs cycle, thus increasing ATP production and insulin secretion. Patients with mutations in this gene have a unique form of CHI characterized by exercise-induced hypoglycemia (20). Inactivating mutations in hydroxyacyl-CoA dehydrogenase (HADH) also cause CHI and have been shown to do so by regulating insulin secretion in a K\textsubscript{ATP} channel–independent fashion (21) and by preventing HADH-mediated inhibition of GLUD1 in the \(\beta\) cell (22). Glucagon-like peptide 1 (GLP1), glucose-dependent insulinotropic peptide (GIP), glucagon, somatostatin, and other signals not shown in this simplified diagram regulate critical amplifying pathways downstream from the CHI-related proteins.
what divergent phenotypes. In many CHI patients, the etiology is still unknown, suggesting that mutations in other genes can cause a similar phenotype.

The most common form of CHI, and the one studied by Henquin and colleagues (2), is caused by inactivating mutations in either of the two genes encoding the subunits of the β cell KATP channel — ABCC8, which encodes sulfonylurea receptor 1 (SUR1), and KCNJ11, which encodes the inward-rectifier potassium channel (Kir6.2). Channel inactivation in these patients results in membrane depolarization and opening of voltage-gated calcium channels, which triggers inappropriate insulin exocytosis. In some patients, abnormal β cells are localized to a discrete focus consisting of a conglomerate of huge, metabolically active islets, with β cells outside of this region showing small nuclei and decreased cytoplasm, suggestive of suppressed function. The unique genetic etiology of this focal form of disease was defined in 1996 (7, 8) and consists of a paternally inherited recessive KATP gene mutation and somatic loss of heterozygosity in a β cell precursor (Figure 2). Such patients can be cured by resection of the lesion. All other genetic mutations in ABCC8 and KCNJ11 result in diffuse forms of CHI, in which all β cells are abnormal. Individuals with this condition often require subtotal pancreatectomy, which is frequently followed by either persistent hypoglycemia or diabetes, depending on the extent of the pancreatectomy and the severity of the mutation.

Functional defects in CHI β cells: prior state of the art

The study by Henquin and colleagues (2), which characterized the in vitro kinetics of insulin secretion by pancreatic fragments obtained at the time of therapeutic pancreatectomy from six patients with diffuse CHI and 18 patients with focal CHI, expands upon previous in vivo and in vitro findings in patients with CHI. Although multiple studies have described the electrophysiological characteristics of affected β cells (9, 10), only a few have examined in vitro insulin secretion in response to secretagogues, and all of these were of limited scope due to scarcity of tissue (11–13). Aynsley-Green demonstrated some increase in insulin secretion by β cells from CHI patients when glucose levels were increased from 0 to 4 mmol, but no further increase at higher concentrations (11). About a decade later, Kaiser and colleagues used static incubations to study pancreatic tissue from five patients with CHI, demonstrating that while stimulators of cAMP production and modulators of the PI3K/PKC pathway stimulated insulin release, glucose did not (12). Secretion was shown to be calcium dependent and partially suppressed by epinephrine or somatostatin. Although this study was performed before the discovery of the genes encoding the KATP channel subunits, one patient was subsequently found to be homozygous for the ABCC8 p.R836* mutation, while three others, all from the same large Arab family, were shown to be homozygous for the ABCC8 c.950delC mutation. Subsequently, Otonkoski and colleagues reported poor responsiveness to glucose and partial suppression with somatostatin in pancreases from six patients with CHI, using human fetal islets for comparison (13).

Functional defects in CHI β cells: novel findings

All previous in vitro studies of CHI-derived pancreatic islets lacked optimal controls, since age-matched normal human islets are not available. Henquin and colleagues solved this problem by using β cells isolated from outside focal lesions as controls (2). Although β cells outside the focal lesion are functionally suppressed in vivo due to increased insulin secretion and hypoglycemia, being homozygous for recessive muta-
tions, they are expected to function normally once outside the patient. Indeed, Henquin and colleagues show that a short period of incubation in stimulatory glucose concentrations is sufficient to restore their responsiveness (2). That being said, the authors are correct in advising caution, since this recovery may not be complete. Using these control islets and a sensitive perfusion system that allowed detailed analysis of secretory dynamics, Henquin and colleagues demonstrated conclusively that CHI islets hypersecrete insulin even at very low glucose levels. Additional findings, such as minimal or no insulin response to glucose or the K\textsubscript{ATP} channel blocker tolbutamide, marked response to stimulators of cAMP, and variable responses to different amino acids were consistent with previous reports and current models of the control of insulin secretion. The finding that β cells from both patients with focal CHI and those with diffuse CHI respond similarly demonstrates that, although the imprinted genes in the distal arm of chromosome 11 may affect β cell replication (14), they do not affect insulin secretion. This finding is novel, albeit expected, since focal CHI and diffuse CHI are clinically similar (15).

In addition to the aforementioned findings, which might have been predicted from previous work, Henquin and colleagues made some unexpected observations (2). Acute elevation of glucose from 1 to 15 mmol/l resulted in transiently increased insulin secretion in some CHI islet preparations. This was unexpected, since this acute insulin response to glucose is thought to be mediated through the K\textsubscript{ATP} channel. Also unexpectedly, insulin secretion decreased during high glucose infusion in some studies, primarily those in which forskolin, which raises cAMP levels, was administered simultaneously. That the transient peak insulin secretion is not mediated through the K\textsubscript{ATP} channel is not entirely unexpected, since some children with CHI do show a brief increase in insulin secretion following glucose stimulation (16). This could be due to further membrane depolarization; however, this seems unlikely since electrophysiological studies in similar patients do not show a glucose effect on β cell calcium currents (9). Alternatively, glucose may be transiently acting downstream of membrane depolarization, stimulating amplying pathways. The decrease in insulin secretion during perfusion with 15 mmol glucose is also unexpected, since continuous exposure to high glucose levels is expected to result in continuously increased insulin secretion, mediated largely by K\textsubscript{ATP} channel-independent pathways. This surprising observation is not explained by Henquin and colleagues (2), but may reflect idiiosyncrasies of the experimental system, since a similar decrease was less evident in insulin ramp experiments or in the absence of forskolin.

Another unexpected finding was the paradoxical response to diazoxide seen in some, but not all, patients. As a K\textsubscript{ATP} channel activator, diazoxide would be expected to be without effect in patients with either ABCC8 or KCNJ11 mutations, as these mutations were thought to either result in permanently closed K\textsubscript{ATP} channels or to entirely prevent protein expression and/or insertion into the plasma membrane. Interestingly, Henquin and colleagues saw a response to diazoxide in one of three patients with mutations that introduced stop codons before the region of ABCC8 that encodes the first nucleotide-binding domain (2), far proximal to the putative potassium channel opener binding site (17). This suggests that the paradoxical response to diazoxide is likely to be an off-target effect. A similar paradoxical response was seen with pinacidil, a K\textsubscript{ATP} channel opener with different channel specificity. While this speaks against an off-target effect, it does not entirely eliminate this possibility. Unfortunately, future detailed studies in human CHI β cells are unlikely, given how difficult it is to obtain appropriate samples. Although, as Henquin and colleagues state (2), this paradoxical response has not been reported in animal models of CHI, this can be tested directly using identical experimental protocols. Furthermore, it is not known whether a similar response is present in vivo, since acute response studies have not been reported in CHI patients with K\textsubscript{ATP} channel mutations.

Conclusion

Given the rarity of CHI and the technical difficulty in obtaining sufficient normal and abnormal tissue, it is not surprising that it took Henquin and colleagues ten years to accumulate sufficient data for their publication (2). The major findings reported here confirm previous findings in humans and in animal models. The novel and unexpected discoveries are not entirely explained, and additional studies, presumably using animal models or in vivo studies in humans, are needed to arrive at a satisfying mechanistic explanation. Nevertheless, this experimental tour de force represents a milestone in β cell research, as it made use of a human inherited condition to validate and expand on models of β cell function largely established in rodent models.

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Address correspondence to: Benjamin Glaser, Endocrinology and Metabolism Service, Hadassah Medical Center, Jerusalem, 91120, Israel. Phone: 972.2.6776599; Fax: 972.2.6437940; E-mail: beng@cc.huji.ac.il.

Atherosclerosis is a chronic inflammatory disease of the arterial walls that often leads to myocardial infarction and/or stroke. Hypercholesterolemia and an imbalance of peripheral leukocyte counts, leading to arterial leukocyte infiltration, are considered independent risk factors for atherosclerosis. However, in this issue of the *JCI*, Murphy and colleagues identify a mechanistic link between hypercholesterolemia, leukocytosis, and the subsequent development of atherosclerotic lesions in mice. These findings could pave the way for the development of novel treatment strategies to control leukocyte homeostasis and atherosclerosis.

Atherosclerosis is a chronic inflammatory disease of the arteries that increasingly threatens human health worldwide as a result of its debilitating, and sometimes life-threatening, consequences, which include limb ischemia, myocardial infarction, and stroke. Identification of novel therapeutic targets is needed to put the brakes on the global increase in the number of cases of atherosclerosis-related diseases.

Two major factors contributing to the pathophysiology of atherosclerosis are hyperlipidemia and inflammation (1). Hyperlipidemia, in particular hypercholesterolemia, is regarded as an independent risk factor in the development of ischemic heart disease, including myocardial infarction (1, 2). Epidemiologic studies have established a strong correlation between elevated total cholesterol levels in serum and morbidity and mortality from myocardial infarction (2). Elevated numbers of circulating neutrophils and monocyes have also been shown to be predictive of cardiovascular events independent of serum cholesterol levels (3, 4). Consistent with this, recent data from mouse models has provided evidence of a direct correlation between the number of circulating and lesional neutrophils or monocytes and atherosclerotic lesion size (5, 6).

In this issue of the *JCI*, Murphy and colleagues have identified ApoE on hematopoietic stem and multipotent progenitor cells (HSPCs) as part of an ABC transporter–mediated efflux pathway and suppressor of proliferation, providing a mechanistic link between hypercholesterolemia, leukocytosis, and the subsequent development of atherosclerotic lesions in mice (ref. 7 and Figure 1). These data have the potential to open the door to the development of novel options for treating atherosclerosis.

### ApoE controls HSPC proliferation and atherosclerosis development

The most commonly used mouse models of atherosclerosis involve feeding a high-fat diet to mice lacking either ApoE or LDLR. Mice lacking ApoA1 (a major component of HDL) do not develop atherosclerosis, but do manifest hypercholesterolemia. Murphy and colleagues report that ApoE−/− mice fed a high-fat diet responded with pronounced proliferation of HSPCs, neutrophilia, and monocytosis, whereas LDLr−/− and ApoA1−/− mice exhibited modest increases in these parameters, suggestive of a specific role for ApoE in suppressing HSPC proliferation (7).

Subsequent analyses showed that ApoE was expressed on the surface of HSPCs and that its expression was further upregulated after treatment with liver X receptor (LXR) activators (7), which have previously been shown to reduce both HSPC proliferation and atherosclerotic lesion formation (8, 9). Moreover, Murphy and colleagues determined that HSPCs themselves secreted ApoE, which became anchored to proteoglycans on the cell surface (7). There it was shown to interact with ABCA1, which transports excessive cholesterol from membranes to nascent HDL particles, and ABCG1, which transports cholesterol to mature HDL particles, thereby controlling intracellular cholesterol levels. The authors then showed that in HSPCs lacking ApoE, cholesterol efflux pathways were disrupted and cholesterol accumulated intracellularly, resulting in increased responsiveness to the hematopoietic growth factors IL-3 and GM-CSF (Figure 1). The net effect was an increase in HSPC proliferation, neutrophilia, and monocytosis, with accelerated atherosclerosis. Thus, a proteoglycan-bound reservoir of ApoE on HSPCs controls their proliferation and circulating leukocyte numbers.

In a therapeutic experiment, Murphy and colleagues injected ApoE−/− mice with cholesterol-poor phospholipid/ApoA-I complexes as cholesterol acceptors, thereby restoring normal HDL proliferation and reducing the expansion of circulating neutrophils and monocytes (7). All the data generated by Murphy and colleagues provide convincing evidence of a mechanistic link between hypercholesterolemia and leukocytosis, and indicate that this contributes to the development of atherosclerotic lesions in mice.

### Keeping the ill alliance of lipids and leukocytosis in check

To fully appreciate the importance of the data provided in this study (7) and to be able to translate these data into the develop-