

Lessons in human biology from a monogenic pancreatic β cell disease

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J Clin Invest. 2011;121(10):3821-3825. <https://doi.org/10.1172/JCI60002>.

Commentary

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.

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work may well be reduced in favor of meaningful, objective data. Just as importantly, these gene expression patterns may help refocus research efforts to understand the pathogenesis of IBD and the mechanisms of treatment resistance.

Implications

The fact that the gene expression signatures identified for AAV and SLE (10), which were very similar to those used by Lee and colleagues (9), could be used to divide normal controls into the same two groups that it did the patients with these various diseases indicates that these gene expression signatures stratify something beyond just a propensity to a particular disease. This suggests that these data indicate some preexisting set point for the inflammatory response that may be independent of any specific disease. We speculate that this non-Gaussian (bimodal) distribution of the population indicates that, in the past, natural selection favored the coordinated increase in gene expression for enhanced immunity and that this pattern of gene expression is now associated with more severe disease in IBD, SLE, and AAV vascular-type inflammation and perhaps

other autoimmune diseases. Coordinating future studies with genome-wide surveys of genetic polymorphisms in larger populations may help identify the master regulator(s) of this particular gene expression signature; again, the bimodal distribution may hint that the number of these master regulators is smaller than expected.

In conclusion, if follow-up studies show that prestratifying IBD patients for different treatment strategies based on CD8⁺ T cell gene expression signatures can lead to improved outcomes, management of IBD will take a giant leap forward. Furthermore, the general approach of Lee et al. (9) may become a useful paradigm for managing potentially hazardous immunotherapies for this and other autoimmune diseases.

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Lessons in human biology from a monogenic pancreatic β cell disease

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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-

Conflict of interest: The author has declared that no conflict of interest exists.

Citation for this article: *J Clin Invest*. 2011; 121(10):3821-3825. doi:10.1172/JCI60002.

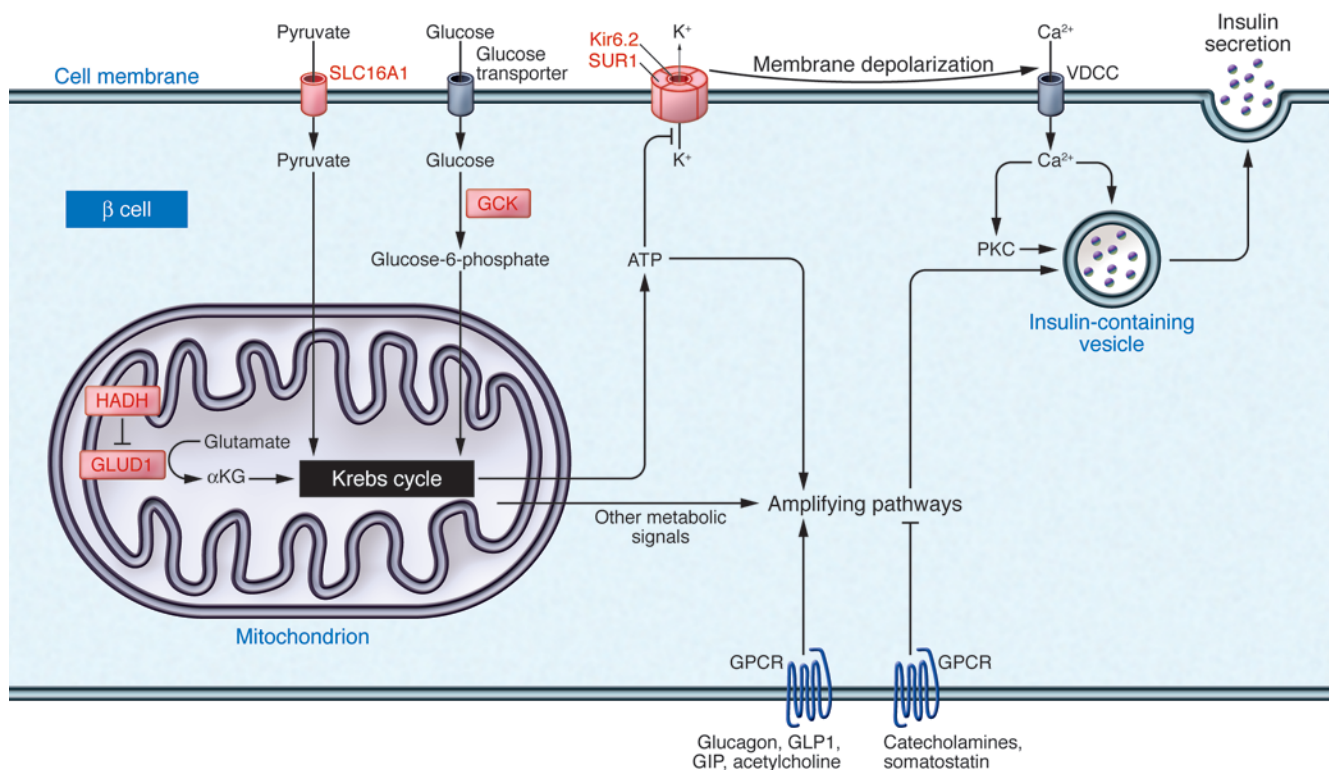


Figure 1

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_{ATP} channel activity and continuous membrane depolarization regardless of glucose levels. Activating mutations in glucokinase (GSK) 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 α -ketoglutarate (α 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 β 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_{ATP} channel-independent fashion (21) and by preventing HADH-mediated inhibition of GLUD1 in the β 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.

cally has a different mutation and studies are done at different ages, under different experimental circumstances, and after different environmental exposures. Patients, or their guardians, frequently do not agree to perform detailed in vivo studies unless they are clinically required, and relevant tissues are often not available for study. These issues make such studies infinitely more difficult, yet because of the limitations of alternative approaches, they are absolutely critical to advancing our understanding of human (as opposed to rodent) physiology.

In this issue of the *JCI*, Henquin and colleagues describe their attempts to decipher the complexities of human β cell physiology through detailed in vitro physiologic analysis of tissues obtained from 24 patients with congenital hyperinsulinism (CHI) (2), a rare genetic disorder that is essentially the

opposite of diabetes, since it is characterized by hypoglycemia, not hyperglycemia (3). CHI is no less relevant to β cell biology than the monogenic forms of diabetes, which are extremely difficult to study, since pancreatic tissue from individuals with these conditions is almost never available. As the most comprehensive study to date using pancreatic tissue obtained from patients with genetically defined CHI, the work of Henquin and colleagues represents a milestone in β cell research (2).

CHI: the basics

CHI is a clinically and genetically heterogeneous disorder typically diagnosed in the newborn period (3). Prior to the discovery of the precise genetic etiology of the most common genetic form of this disease in 1995 (4), the pathophysiology and clinical features

were hotly debated, and the clinical syndrome was referred to by different descriptive names. These included nesidioblastosis, which described the histologic picture that was subsequently shown to be nonspecific (5); β cell dysmaturation syndrome, since some histologic and functional characteristics of the disease are reminiscent of the fetal pancreas (6); persistent hyperinsulinism of infancy (PHII); hyperinsulinemic hypoglycemia, familial (HHF); and CHI. The latter, used by Henquin and colleagues (2), seems to be most popular, although the OMIM website uses HHF (OMIM 256450).

Once thought to be a single entity, it is now known that CHI can be caused by mutations in at least six different genes (*ABCC8*, *KCNJ11*, *GSK*, *GLUD1*, *HADH*, and *SLC16A1*), each of which affects the β cell differently (Figure 1), resulting in some-

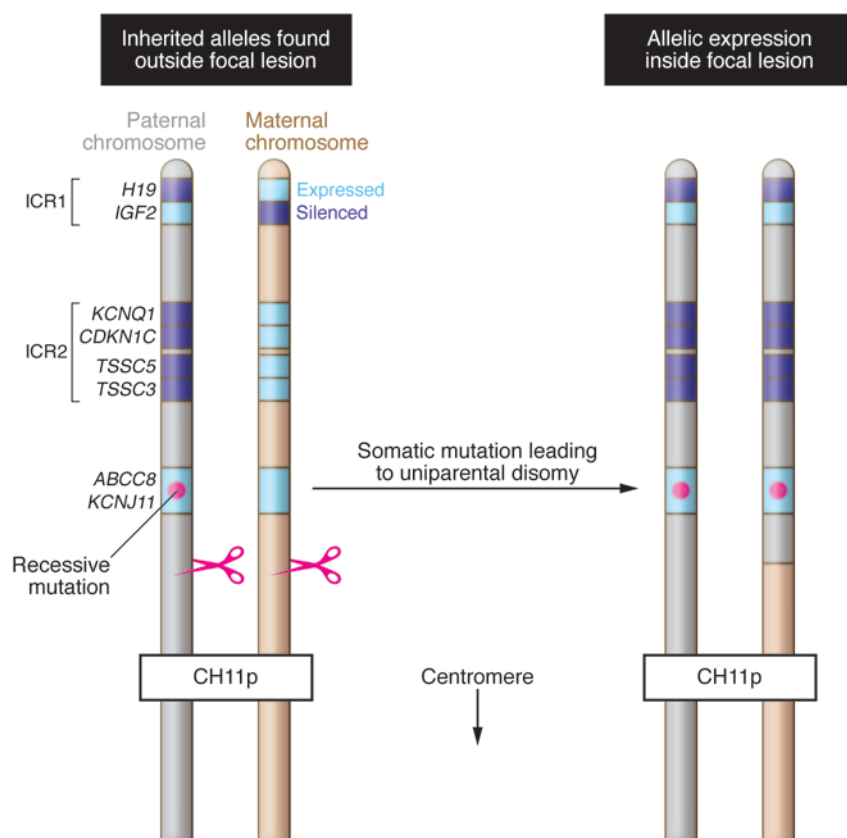


Figure 2

Schematic representation of the molecular defect resulting in focal CHI. On the left is shown a schematic representation of the paternal and maternal copies of the distal short arm of chromosome 11, inherited by the fetus at risk for focal CHI and present in all cells except those within the focal lesion. A single *ABCC8* or *KCNJ11* recessive mutation is inherited on the paternal allele (red dot). Distal to the *ABCC8/KCNJ11* locus, there are two imprinted regions. The relevant genes are shown, and the expressed allele for each is shown in light blue. During fetal development, a somatic mutation occurs in a β cell precursor, which results in loss of the maternally inherited allele and duplication of the paternally inherited allele, as depicted on the right. In this cell and all of its progeny, both *ABCC8* or *KCNJ11* alleles are mutated, resulting in insulin hypersecretion. In addition, two copies of growth-promoting genes such as *IGF2* are expressed, while growth-inhibiting genes such as *H19* and *CDKN1C* are not expressed at all. The result is a proliferating lesion consisting of β cells lacking a functioning K_{ATP} channel and thus hypersecreting insulin at low glucose levels.

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 K_{ATP} 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 K_{ATP} 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 K_{ATP} 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 heterozygous 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_{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_{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_{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 amplifying 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_{ATP} channel-independent pathways. This surprising observation is not explained by Henquin and colleagues (2), but may reflect idiosyncrasies of the experimental system, since a similar decrease was less evident in glucose 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_{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_{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_{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_{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.

Acknowledgments

I would like to thank Yuval Dor for reviewing the manuscript and providing very helpful comments and suggestions.

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ApoE controls the interface linking lipids and inflammation in atherosclerosis

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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 monocytes 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 multipotential progenitor cells (HSPCs) as part of an ABC transporter-mediated cholesterol 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 HSPC 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 devel-

Conflict of interest: Christian Weber and Oliver Soehnlein are stakeholders of Carolus Therapeutics Inc.

Citation for this article: *J Clin Invest*. 2011; 121(10):3825–3827. doi:10.1172/JCI60457.