would have minimal roles in long-term regulation, and it is possible that these differences reflect signaling through different neuronal subpopulations. Thus, perhaps neurons projecting to ganglia that regulate sympathetic tone signal through different pathways than those projecting rostrally to regulate feeding behavior. The infusions employed in the current study were delivered to the medial basal hypothalamus, but with this approach it is not possible to know which neuronal subpopulations were targeted.

Another issue in interpreting the data of Buettner *et al.*[1] is that the experimental model employed attempts to isolate 'pure' leptin action by clamping glucose and insulin levels. This model does not fully reflect the effect of leptin in a physiologic context. Leptin treatment leads to suppression of food intake and to a rapid fall in insulin as well as glucose. Because insulin stimulates lipogenesis, a decline in insulin would result in less lipogenesis, and this effect would be augmented by a decline in glucose abundance, which would reduce substrate availability. In such a context, the effects of leptin on lipogenesis might be much less prominent.

The findings of Buettner *et al.*[1] highlight the complexity of leptin signaling pathways, which involve multiple neuronal populations and multiple effector pathways. The original simple view that leptin acts to reduce energy stores by inhibiting food intake has evolved as the effect of leptin on energy expenditure has become apparent.

This study makes it clear that leptin can directly regulate adipose tissue through a complex relay that begins in the medial basal hypothalamus and extends to the sympathetic nervous system, which, in turn, alters endocannabinoid tone in the adipocytes—ultimately leading to changes in lipid metabolism.

Fully understanding this relay will require specifying the identity of both target neurons and the induced cellular responses.

1. Buettner, C. *et al. Nat. Med.* **14**, 667–675 (2008).
2. Ahima, R.S. *et al. Nature* **382**, 250–252 (1996).
3. Myers, M.G., Cowley, M.A. & Munzberg, H. *Annu. Rev. Physiol.* **70**, 537–556 (2008).
4. Bjorbaek, C. & Kahn, B.B. *Recent Prog. Horm. Res.* **59**, 305–331 (2004).
5. Guo, K. *et al. Endocrinology* **148**, 3987–3997 (2007).
6. Halaas, J.L. *et al. Proc. Natl. Acad. Sci. USA* **94**, 8878–8883 (1997).
7. Cone, R.D. *et al. Int. J. Obes. Relat. Metab. Disord.* **25** Suppl 5, S63–S67 (2001).
8. Bates, S.H. *et al. Nature* **421**, 856–859 (2003).
9. Gong, L. *et al. Endocrinology* published online, doi:10.1210/en.2007-0945 (10 April 2008).
10. Hill, J.W. *et al. J. Clin. Invest.* **118**, 1796–1805 (2008).

Keeping blood clots at bay in sepsis

Cornelis van 't Veer & Tom van der Poll

Clearance of platelets by the liver can help counteract the dangerous blood coagulation that can occur during sepsis. The mechanism involves clearance of platelets through the liver's Ashwell receptor, which binds to platelet glycoproteins altered by sepsis-causing bacteria (pages 648–655).

The glycan branches of the body's ubiquitous glycoproteins, which decorate many cell types, are not naked. They often sprout sialic acid (*N*-acetylneuraminic acid) at their termini in a process named sialylation. Sialylation can be crucial for the function of glycoproteins and their rate of clearance from the body.

Glycoproteins with these branches devoid of sialic acid, dubbed asialoglycoproteins, can be rapidly cleared by the hepatic asialoglycoprotein receptor named after its co-discoverer, Gilbert Ashwell[1] (see interview, page 608). Asialoglycoprotein receptors have an established function in drug metabolism by virtue of their capacity to remove exogenously administered asialoglycoproteins from the circulation. But, until recently, endogenous ligands for this receptor family had not been discovered.

Cornelis van 't Veer and Tom van der Poll are at the Centre for Infection and Immunity Amsterdam and the Centre for Experimental and Molecular Medicine, Academic Medical Centre, University of Amsterdam, Meibergdreef 9, Room G2-129, 1105 AZ Amsterdam, The Netherlands.
e-mail: c.vantveer@amc.uva.nl or t.vanderpoll@amc.uva.nl

In this issue, Grewal *et al.*[2] uncover the ligand for the Ashwell receptor while providing insight into the pathogenesis of sepsis. They provide evidence that the sialidase activity of pathogens that cause sepsis may alter endogenous glycoproteins, thereby unmasking a ligand for the Ashwell receptor on hepatocytes. As a result, the receptor mediates the clearance of platelets and reduces peripheral blood platelet counts—a process that seems to protect the host from disseminated intravascular coagulation (DIC), a feared complication of sepsis.

These findings not only provide insight into how reduced platelet count (thrombocytopenia) develops during severe infection, but also identify a role for hepatocytes in the regulation of the procoagulant response to sepsis.

Patients with sepsis almost invariably show evidence for activation of the coagulation system. When coagulation is insufficiently controlled, DIC may evolve, resulting in a clinical syndrome that involves both widespread microvascular thrombosis (because of excessive clotting) and enhanced bleeding (because of depletion of clotting factors).

In a previous study, the authors became intrigued by the role of the Ashwell receptor in sepsis after observing that desialylated platelets and von Willebrand factor, a blood glycoprotein involved in hemostasis, can be removed from the circulation by asialoglycoprotein receptors[3]. To show this, they examined mice that are unable to adequately decorate their glycoproteins with sialic acid owing to a deficiency of ST3Gal-IV, a sialyl transferase[3]. These mice have low platelet counts and low plasma levels of von Willebrand factor caused by enhanced asialoglycoprotein receptor–mediated clearance.

In the current study, the authors elaborate on these earlier findings[2]. They examined mice deficient in either one of the two subunits of the Ashwell receptor, the asialoglycoprotein receptor (Asgr)-1 and Asgr-2. Whereas these subunits can interact in different combinations, the high-affinity binding receptor is an Asgr-1–Asgr-1–Asgr-2 trimer that grasps triplets of nonsialylated terminal galactose residues[4] (**Fig. 1**).

The authors show that the reduction in von Willebrand factor abundance in ST3Gal-IV–deficient mice is solely dependent on Asgr-1 and that depletion of platelets decorated with asialoglycoproteins is dependent on both Asgr-1 and Asgr-2 (ref. 2). The authors

reasoned that ST3Gal-IV deficiency may model aspects of sepsis, given that several human pathogens express sialidases. Might platelets therefore be desialylated in bacterial sepsis[2]?

To provide evidence for this challenging hypothesis, the authors used a sepsis model with *Streptococcus pneumoniae* and conducted several reciprocal experiments[2]. They showed that sepsis caused by wild-type *S. pneumoniae*, but not by an isogenic mutant *S. pneumoniae* strain lacking the sialidase enzyme neuraminidase-A, resulted in an increase in asialoglycoprotein-covered platelets in the circulation[2]. Wild-type mice infected with wild-type *S. pneumoniae* developed thrombocytopenia owing to enhanced platelet clearance; both Asgr-1 and neuraminidase-A seemed to be essential for this phenomenon. Asgr-1–deficient mice did not develop thrombocytopenia after infection with wild-type pneumococci, whereas wild-type mice did not show thrombocytopenia after infection with neuraminidase-A–deficient *S. pneumoniae*.

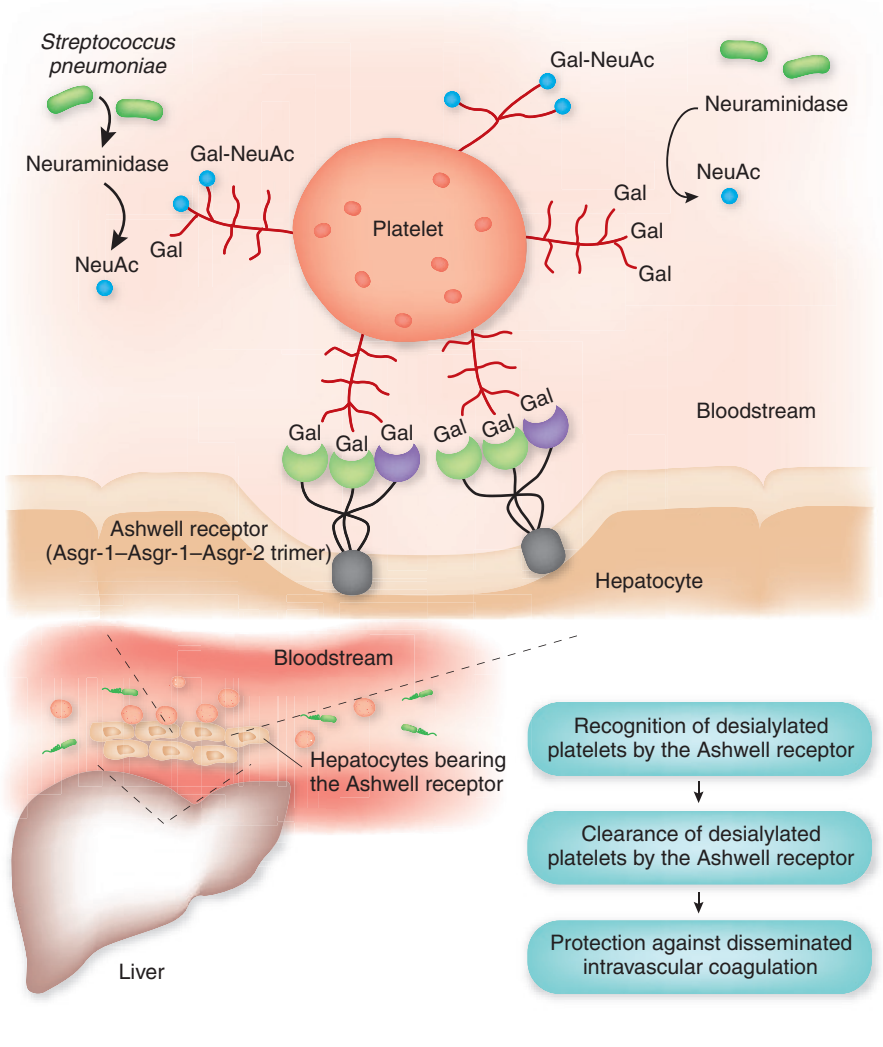
Depletion of asialoglycoprotein-covered platelets by the Ashwell receptor created a nonthrombotic phenotype that protected against DIC, that is, wild-type mice infected with wild-type *S. pneumoniae* survived longer and had less evidence of DIC when compared with either Asgr-1– or Asgr-2–deficient mice infected with wild-type *S. pneumoniae* or wild-type mice infected with neuraminidase-A–deficient pneumococci.

Sepsis is a complicated disease, and the new studies must be considered in the context of other aspects of physiology. Whereas Grewal *et al.*[2] demonstrate a beneficial effect of reduced platelet counts, in patients with sepsis thrombocytopenia is associated with a higher mortality[5]—suggesting that once DIC occurs, a drop in platelet counts represents a failed attempt of the host to limit excessive coagulation. Although platelets have a major role in coagulation, DIC is probably also caused by a concurrent disturbance of several pathways designed to maintain a normal hemostatic balance, including the tissue factor pathway (which activates coagulation), antithrombin and the protein C system (which inhibits coagulation), and the fibrinolytic system[6]. Moreover, DIC can also occur in sepsis caused by pathogens that do not express sialidases; in these infections, the mechanism implicated by Grewal *et al.*[2] cannot be involved.

The role of neuraminidases in pneumococcal infection is likewise complex. Neuraminidase A promotes pneumococcal survival in the respiratory tract and bloodstream and may mediate colonization of the nasopharynx, both benefiting the pathogen[7,8], whereas Grewal *et al.*[2] point to a host protective role for neuraminidase A during infection with *S. pneumoniae*. Notably, the investigators administered *S. pneumoniae* via intraperitoneal injection, bypassing the epithelial barrier and host resistance normally encountered by pathogens in the lung[2]. Additionally, sialylation of ligands for E-, L- and P-selectin is crucial for homing and extravasation of leukocytes, and sialylation masks mannose antigens on host cells for mannose-binding lectin, thereby protecting against complement activation[9,10].

Thus, in addition to the effect on platelets, desialylation by neuraminidase may affect other parts of the host response to pneumococcal infection. Notably, neuraminidase inhibitors used as anti-flu agents (for example, oseltamivir) in theory could interfere with the protective effect of the Ashwell receptor, which could affect the course of postinfluenza pneumococcal pneumonia.

Grewal *et al.*[2] have shown that the Ashwell receptor, by removing asialoglycoprotein-covered platelets from the bloodstream in a mouse model of pneumococcal sepsis, mitigates the subsequent development of severe coagulopathy and death. It will be interesting to uncover the particular asialoglycoprotein(s) on platelets involved in this phenomenon and to find whether human platelets display Ashwell receptor ligands under desialylating conditions.



Kim Caesar

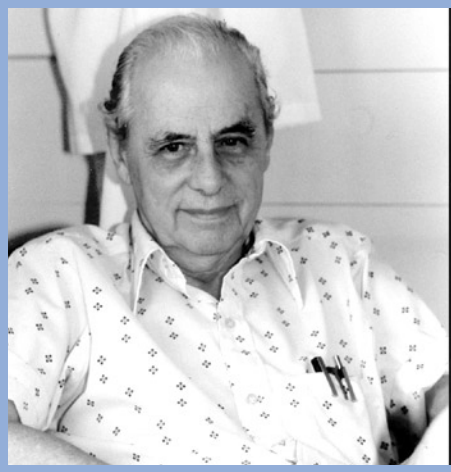
Figure 1 Eliminating platelets during sepsis. During sepsis with *S. pneumoniae,* sialic acid (NeuAc) is removed from galactose (Gal) residues at the surface of circulating platelets by neuraminidase A, a sialidase expressed by the pathogen. Grewal *et al.*[2] show that this process unmasks the ligand for the Ashwell receptor (an Asgr-1–Asgr-1–Asgr-2 trimer), resulting in binding to hepatocytes and platelet clearance. The ensuing reduction in platelet counts at the onset of sepsis protects the host against the development of disseminated intravascular coagulation.

Furthermore, at present it remains unclear whether blocking the Ashwell receptor—for instance, with synthetic high-affinity ligands—may diminish thrombocytopenia once full-blown DIC has developed and whether this could have a beneficial effect in severe sepsis.

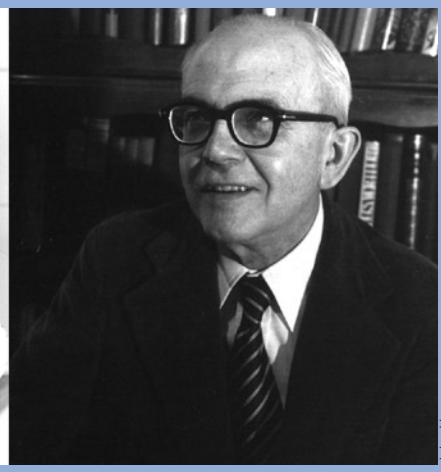
1. Ashwell, G. & Harford, J. *Annu. Rev. Biochem.* **51**, 531–554 (1982).
2. Grewal, P.K. *et al. Nat. Med.* **14**, 648–655 (2008).
3. Ellies, L.G. *et al. Proc. Natl. Acad. Sci. USA* **99**, 10042–10047 (2002).
4. Stockert, R.J. *Physiol. Rev.* **75**, 591–609 (1995).
5. Ogura, H. *et al. Shock* **28**, 411–417 (2007).
6. Schouten, M., Wiersinga, W.J., Levi, M. & van der Poll, T. *J. Leukoc. Biol.* **83**, 536–545 (2008).
7. Kadioglu, A., Weiser, J.N., Paton, J.C. & Andrew, P.W. *Nat. Rev. Microbiol.* **6**, 288–301 (2008).
8. Manco, S. *et al. Infect. Immun.* **74**, 4014–4020 (2006).
9. Magnani, J.L. *Arch. Biochem. Biophys.* **426**, 122–131 (2004).
10. Fujita, T., Matsushita, M. & Endo, Y. *Immunol. Rev.* **198**, 185–202 (2004).

Gilbert Ashwell: sweet on science

In 1974, Gilbert Ashwell and Anatol Morell discovered a receptor in the liver that recognizes particular glycoproteins, dubbed asialoglycoproteins. We asked Ashwell about his discoveries and what he thinks of the study by Grewal *et al.*[1] in this issue, which suggests that the receptor is involved in regulating sepsis.



Gilbert Ashwell



Anatol Morell

John Hanover

How did you discover the receptor?

It came by accident, as many things do in science. I was on sabbatical at Columbia University in 1965–1966, and my dear friend Anatol G. Morell and his wife, Halina, frequently invited me to dinner, as I was alone in New York—my family was in Washington. During one of those times, Anatol, who was at the Albert Einstein College of Medicine, brought up that he had a problem determining the half-life of the glycoprotein he was working on, ceruloplasmin, a copper-carrying protein. Since my background was more in carbohydrates, I suggested that we label a terminal galactose with a long-lasting tritium isotope to determine the serum half-life.

This material, when injected into a rabbit, rapidly disappeared from the serum within five to ten minutes and was recovered essentially complete in the liver. The critical problem then was the demonstration that galactose was the unique sugar required for hepatic recognition by the then–unknown carbohydrate-binding protein.

From there we went on to isolate and chemically characterize the appropriate receptor as the first mammalian lectin. We both feel that if this receptor is to be identified, other than as an asialoglycoprotein receptor, a more appropriate name would be the Ashwell-Morell receptor.

What were some of the early implications of the finding?

For years it had been known that hormones were inactive if they had been desialyated in preparation; with this finding it became immediately clear what happened: the hormones never got to their target organ—they were disposed of in the liver. Since then, people have used the receptor in experiments to deliver drugs specifically to the liver. I worked for years and years to finally convince people that the carbohydrates were more important than just glucose.

What do think of the study of Marth and his colleagues in this issue?

I was dumbfounded. I was so completely taken aback, because I had been working on this for over 30 years, trying find out the real biological function of this protein. I, and others, had worked with the knockout mice and found that they had perfectly normal lives, as far as we could tell. We had figured originally that we had discovered the normal mechanism for the turnover of serum glycoproteins, and when I found out that wasn't right, I was very despondent. Hence my delight in learning of Dr. Marth's success; this was the first real evidence of physiological function.

You still go into the lab every day. What are you working on now?

I still work, as a guest worker, in the lab of John Hanover at the National Institutes of Health. I have been involved in studying changes in carbohydrate metabolism in various mutant forms of *C. elegans*. With the exception of the last few weeks, when I've been combating a neck problem, I get to the lab at seven and work until noon. I will be 92 in July, so I feel I have a right to take only part of the day. I can't stay away.

1. Grewal *et al. Nat. Med.* **14**, 648-655 (2008).