Synthetic human antibody libraries are based on randomized complementarity determining regions (CDRs) of the variable domains of light and heavy chains (see the figure on the left on page 2889). Analogous to chemical libraries, synthetic human antibody libraries allow iterative optimization by simultaneous and sequential selections of randomized CDRs, ultimately providing selectable solutions for many challenging antigens (see the figure on the right on page 2889).

Facing rapid progress of human mAb engineering and evolution through phage display technology, hybridoma technology has maintained a competitive position, in particular the development of transgenic mice expressing human antibodies. However, the work by Lee and colleagues reiterates the advantage of phage display technology when it comes down to generating human mAbs with highly defined properties.

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REGULATING THE MASTER IRON REGULATOR HEPcidIN

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The inflammatory cytokine IL-6 directly regulates hepcidin through induction and subsequent promoter binding of STAT3. Clarification of the mechanism regulating hepcidin may allow the development of innovative therapeutic interventions for clinical conditions of abnormal iron homeostasis.

Hepcidin is a circulating hormone that plays a central role in iron homeostasis. Increased hepcidin production associated with excess iron or inflammation inhibits iron absorption and iron recycling from macrophages. Conversely, iron deficiency and genetic hemochromatosis are associated with decreased hepcidin production, resulting in increased iron absorption and recycling. The mechanisms regulating hepcidin expression remained largely unknown. Recent studies have shown that a bone morphogenetic protein BMP/SMAD signaling cascade is important for basal regulation of hepcidin transcription.1,2 Although that pathway clarifies the role of hemojuvelin in hepcidin regulation, it does not account for the induction of hepcidin expression in inflammation.

One of the important mediators of inflammation is the cytokine interleukin-6 (IL-6). Upon an inflammatory stimulus, IL-6 is released and binds to a complex of the IL-6 receptor α and gp130. The IL-6 ligand receptor interaction results in the activation of Janus kinases (JAKs) that phosphorylate signal transducers and activators of transcription (STAT) proteins, predominantly STAT3 (see figure). Upon phosphorylation at tyrosine residue 705, STAT3 translocates into the nucleus, where it regulates the transcription of many target genes.1 IL-6 treatment stimulates hepcidin expression in isolated hepatocytes, and administration of IL-6 to human subjects stimulates hepcidin production and results in low serum iron (hypoferremia) in vivo.

In their study in this issue, Wrighting and Andrews intended to clarify the role of IL-6 in the stimulation of hepcidin production associated with inflammation by determining whether IL-6 acts directly to up-regulate hepcidin expression and by elucidating the downstream mechanism of IL-6-mediated hepcidin induction. In a series of elegant experiments, the authors have identified an IL-6-responsive element in the putative hepcidin promoter; demonstrated that IL-6 regulates hepcidin expression through direct binding of STAT3 to the promoter; and, finally, demonstrated that STAT3 is necessary and sufficient to confer IL-6 responsiveness. These observations not only illuminate IL-6 regulation of hepcidin but also suggest that, even in the absence of elevated cytokine levels, aberrations

IL-6 binds to a complex of the IL-6 receptor α and gp130. This results in the activation of Janus kinases (JAKs) that phosphorylate signal transducer and activator of transcription 3 (STAT3). Upon phosphorylation, STAT3 dimers are formed through phosphoYXXQ motifs (Y) and translocate into the nucleus where they regulate hepcidin transcription. Adapted with permission from Heinrich et al®; copyright 2003 The Biochemical Society.
in hepatic STAT3 regulation could lead to increased hepcidin and anemia.

Collectively, these studies indicate the existence of at least 2 signaling pathways involved in the transcriptional regulation of hepcidin: the BMP/SMAD signaling of hemojuvelin and the IL-6/STAT3 signaling of inflammation. This impressive advance in our current understanding implies that, with further progress in this line of research, new pharmacologic approaches could eventually be developed for innovative therapeutic interventions.

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Hiding in plain sight

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Epstein-Barr virus (EBV)–infected B lymphocytes and T cells achieve a homeostatic balance in healthy individuals. Increasing virus-specific cytotoxic T lymphocyte (CTL) numbers by adoptive immunotherapy may be effective as antitumor therapy but not affect the viral load in peripheral blood lymphocytes.

In this issue of Blood, Savoldo and colleagues report on their experience expanding and infusing autologous CTLs targeting EBV in solid organ transplantation (SOT) patients at high risk for EBV lymphoproliferative disease or with established EBV lymphoproliferative disease.

The authors report no barrier to the expansion of EBV-specific cells from patients on posttransplantation immunosuppression but note that although the infused cells expand in vivo, the expansion is much more limited than that achieved in the bone marrow or hematopoietic stem cell transplantation setting, perhaps reflecting availability of lymphoid space in the marrow or hematopoietic stem cell transplantation settings. Both observations are important for the application of adoptive cellular immunotherapy.

Of special interest to those concerned with the nature of viral persistence is the observation that the infusion of cytotoxic T lymphocytes (CTLs) targeting EBV often does not affect viral genome copy number in peripheral blood lymphocytes (see figure)—even when these CTLs are active in killing localized tumor. This likely reflects the very limited viral gene expression in peripheral blood lymphocytes that harbor virus. Although in the laboratory, infection of resting B lymphocytes leads to the outgrowth of lymphoblastoid cell lines expressing many viral genes and continuously proliferating, it is now clear that in healthy EBV-seropositive individuals and even in many patients on immunosuppressive agents or with human immunodeficiency virus infection, EBV genomes are predominantly harbored in resting memory B cells that express few if any viral antigens. This ability to persist in resting cells with limited viral gene expression is part of the explanation for life-long persistence of EBV infection. As the present report indicates, even when EBV CTL numbers are increased by adoptive immunotherapy, genome copy numbers in these cells are not changed. The virus can hide in plain sight. The good news for patients is that adoptive T-cell therapy can be effective against tumor cells expressing a spectrum of antigens even if these virus-infected lymphocytes in the blood evade the CTLs.

Monitoring of EBV load in SOT recipients after adoptive transfer of EBV-CTLs.

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Regulating the master iron regulator hepcidin

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