Synthetic antibodies prey on B cells

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As if taken from a Michael Crichton novel, the evolution of synthetic antibodies that target B cells, the architects and manufacturers of natural antibodies, is described by Lee and colleagues.

At the center of this twist is BAFF, a soluble ligand that mediates B-cell survival and maturation through its receptor BR3. Antagonists of the BR3/BAFF interaction are of considerable interest for the therapy of B-cell malignancies and autoimmune diseases. In their quest for human monoclonal antibodies (mAbs) to BR3 that antagonize the BR3/BAFF interaction with high precision and, at the same time, cross-react with human and murine BR3, Lee and colleagues turn to synthetic human antibody libraries and phage display technology. Not only do they meet their challenge, but they analyze the outcome in meticulous structural detail, providing important insights to antibody/antigen interactions that delineate affinity, specificity, and species cross-reactivity at the molecular level.

Currently, 17 of 18 mAbs approved for therapeutic applications by the Food and Drug Administration (FDA) are derived from murine mAbs generated by hybridoma technology.2 Murine mAbs are highly immunogenic in humans, severely limiting their therapeutic applications and making antibody humanization or at least chimerization mandatory if repeated administration is required. In addition, murine mAbs to human antigens often do not cross-react with the murine antigen, which weakens the reliability of activity and toxicity profiles of mAbs in murine models of human disease. Based on these considerations, the ideal therapeutic mAb is human and cross-reacts with human and murine antigens while maintaining high specificity and strong affinity.

With the concept of phage display technology, the generation of human mAbs with selectable properties, including affinity, specificity, and species cross-reactivity, has become reality. The hallmark of phage display and combinatorial antibody technology is the physical connection of antibody phenotype (protein) and genotype (cDNA), effectively allowing selection rather than screening of mAbs from vast libraries. In addition, and in contrast to hybridoma technology, phage display permits the generation of mAbs from virtually any species whose immunoglobulin genes are known. In particular, large naive and synthetic human antibody libraries have become accessible through phage display technology, yielding the first FDA-approved library-derived human mAb, adalimumab, and dozens more human mAbs at various stages in preclinical and clinical development.2

The antibody molecule, here shown as IgG1, contains 2 identical light chains (purple) and 2 identical heavy chains (green). The light chain consists of 1 N-terminal variable domain (VL) followed by 1 constant domain (CL). The heavy chain consists of 1 N-terminal variable domain (VH) followed by 3 constant domains (CH1, CH2, and CH3). The antigen-binding site results from the convergence of 6 CDRs, 3 provided each by VL and VH. F(ab’)_2 and Fc fragments of the antibody molecule are indicated. Illustration by Paulette Dennis.

Synthetic human antibody libraries used by Lee and colleagues are based on phage-displayed bivalent F(ab’)_2 or monovalent Fab with randomized CDRs. Starting out from bivalent F(ab’)_2 libraries that use avidity to make up for weak affinities, a Fab lead with micromolar affinity to the murine antigen and even weaker cross-reactivity with the human antigen was selected. Iterative optimization strategies that involved both simultaneous and sequential selections of randomized CDRs resulted in a final Fab that bound both murine and human antibody to subnanomolar affinity. Compared with the lead Fab, the final Fab had acquired 13 mutations spread over 4 CDRs (bold). Illustration by Paulette Dennis.
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 through further advancements, 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.

REFERENCES

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. 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. 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...