

Built in part on the foundation of these earlier experiences, the current study reports on a new conditional transformation strategy that emphasizes early clonal selection for  $\beta$ -cell phenotype (Fig. 1). The authors generated immortalized cell lines by transformation of human islet cells with retroviral vectors expressing SV40 large T-antigen (*SV40T*) and human telomerase reverse transcriptase (*TERT*), with both of these genes flanked by loxP sites for later Cre recombinase-based excision. An initial screen tested 271 transformed cell lines for tumorigenicity in severe combined immunodeficiency (SCID) mice, whereas a second screen searched for expression of insulin and four  $\beta$ -cell-enriched transcription factors. Only one of the 253 nontumorigenic cell lines, designated NAKT-15, expressed insulin and the four transcription factors. The authors designed their system such that subsequent Cre-mediated excision of the *SV40T* gene unmasked expression of the *NeoR* gene, allowing selection of *SV40T*-negative cells. Moreover, coexpression of *SV40T* and herpes simplex thymidine kinase (*HSV-TK*) allowed use of ganciclovir as a secondary selection agent. Finally, the viral vector containing *TERT* was designed to coexpress green fluorescent protein (*GFP*), allowing identification and removal of cells that continued to express *TERT* after Cre delivery.

Relative to the parental, transformed NAKT-15 cells, reverted NAKT-15 cells exhibited large increases in expression of insulin and other genes characteristic of mature  $\beta$ -cells. Impressively, insulin secretion from these cells was shown to be regulated by glucose in a dose-dependent manner over a physiologically relevant range, with maximal stimulation of nearly 8-fold at 25 mM glucose relative to 5.6 mM glucose, comparable to the fold response of human islets tested in parallel. Moreover, at an intermediate glucose dose (10 mM), reverted NAKT-15 cells were able to respond to a series of relevant potentiators of the glucose response, including tolbutamide, glucagon-like peptide 1 (GLP-1) and fatty acids, again with levels of response comparable to those of human islets. However, insulin content in reverted NAKT-15 cells was only 40% of that of human islets, and several  $\beta$ -cell-enriched transcription factors, hormone processing enzymes, and secretory granule proteins were 20–40% as abundant. Nevertheless, implantation of approximately 3 million reverted NAKT-15 cells (roughly equivalent to 2,000 islets) under the kidney capsule of diabetic mice resulted in lowering of blood glucose to the normal range for a period of 30 weeks, with no evidence of gross hypo- or hyperglycemic episodes over this time frame.

Does the development of this new reversibly immortalized cell line herald the end of the search for a human  $\beta$ -cell surrogate for diabetes therapy? For diabetes patients and islet researchers who have been repeatedly jilted at the altar as promising new approaches in this field have failed to progress to clinical relevance, such a conclusion would seem foolhardy. However, it is also fair to say that the current study represents as promising an approach to human  $\beta$ -cell expansion as has emerged to date. Further work is now required to confirm the new findings and answer important remaining questions.

Foremost among these is whether a growth-promoting strategy that involves genetic engineering of cell lines with an oncogene will ever be blessed by regulatory agencies such as the US Food and Drug Administration. The authors are certainly aware of this issue, and are to be commended for installation of redundant safeguards (e.g., use of nontumorigenic cells and multiple anti-oncogene selections systems), but can they be certain that all oncogene expression is eliminated? Also, based on the present studies in mice, it can be estimated that about one billion reverted NAKT-15 cells will be needed for therapy of one human diabetic patient, clearly requiring large-scale cell growth. In such a context, will hundreds of population doublings result in somatic mutations, as has been documented previously for cells harboring *SV40T*? Finally, the current studies were performed in

immune-compromised SCID mice, leaving open the question of how reverted NAKT-15 cells will fare when implanted in patients with autoimmune diabetes, including how they will respond to current and future immunosuppressive regimens.

These issues notwithstanding, the current report seems to represent a substantive step forward. An added potential benefit of the study is that the traditional reliance on rodent cell lines for studies of islet biology<sup>10</sup>, forced by the absence of well-differentiated human  $\beta$ -cell lines, can now be reconsidered. If the phenotype of the current line can be sustained and confirmed, its importance as a tool for discovering drugs that enhance insulin secretion in type 2 diabetes could rival its potential utility for treatment of the type 1 disease.

1. Narushima, M. *et al. Nat. Biotechnol.* **23**, 1274–1282 (2005).
2. Shapiro, A.M.J. *et al. N. Engl. J. Med.* **343**, 230–238 (2000).
3. Colman, A. *Sem. Cell Develop. Biol.* **15**, 337–345 (2004).
4. Ferber, S. *et al. Nat. Med.* **6**, 568–572 (2000).
5. Zalzman, M. *et al. Proc. Natl. Acad. Sci. USA* **100**, 7153–7258 (2003).
6. Gershengorn, M.C. *Science* **306**, 2261–2264 (2004).
7. de la Tour, D.D. *et al. Mol. Endocrinol.* **15**, 476–483 (2001).
8. Beattie, G.M. *et al. Diabetes* **51**, 3435–3439 (2002).
9. Kulkarni Schmidt-Kastner, P.S., Heiden, T., Tribukait, B. & Grafstrom, R.C. *Anticancer Res.* **16**, 2681–2686 (1996).
10. Hohmeier, H.E. & Newgard, C.B. *Mol. Cell. Endocrinol.* **228**, 121–128 (2004).

## Antibodies in the breakdown lane

Richard S Blumberg & Wayne I Lencer

**An engineered IgG induces degradation of endogenous IgG, providing a potential therapeutic approach to autoimmune disease.**

Immunoglobulin G (IgG), the most abundant protein in human serum, has long been a target of biotechnological tinkering. For the most part, such efforts have sought to take advantage of the interaction of IgG with one of its

receptors, FcRn, to improve the pharmacokinetics of therapeutic molecules coupled to the immunoglobulin. In this issue, Vaccaro *et al.*<sup>1</sup> take antibody engineering in a new direction. They describe an IgG variant that blocks FcRn function by binding tightly to the receptor and competing with endogenous IgGs, thereby hastening their degradation. This work suggests the potential of antibody Fc engineering in the development of therapeutics for antibody-mediated disorders, including autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus.

IgG is the longest-lived and most highly expressed of the serum proteins, as befits its

Richard S. Blumberg is at Brigham and Women's Hospital and Harvard Medical School, 75 Francis Street, Boston, Massachusetts 02115 USA, and Wayne I. Lencer is at Children's Hospital Medical Center and Harvard Medical School, 300 Longwood Avenue, Boston, Massachusetts, 02115 USA.  
e-mail: rblumberg@partners.org

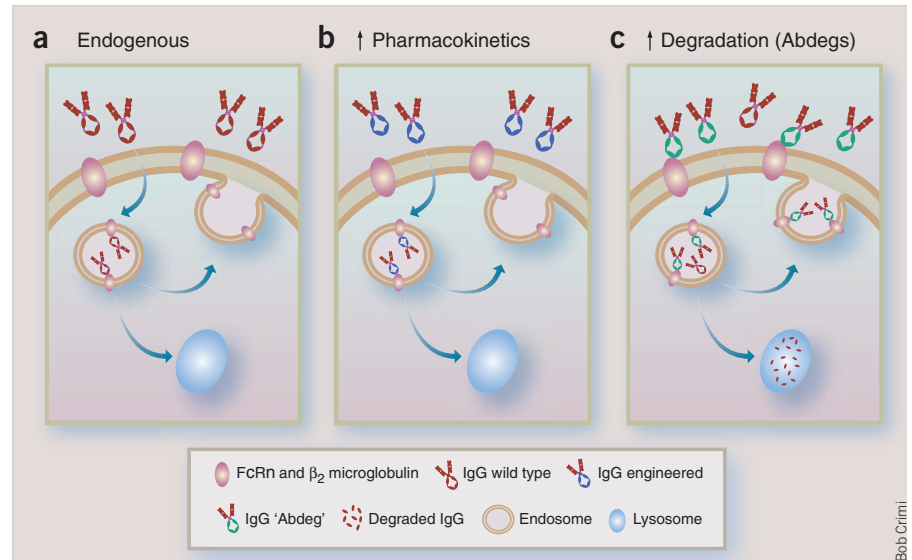
major functions in immunity and immunoregulation. This long half-life is due to the fact that IgG catabolism and, interestingly, transport of IgG across single-layered epithelial barriers are tightly managed by FcRn (n for neonatal). Unlike other Fc receptors, FcRn is structurally related to major histocompatibility complex class I molecules and functions primarily as a trafficking rather than a signaling receptor for IgG<sup>2,3</sup>.

FcRn binds the Fc domain of IgG at acidic pH (<6.0) and releases it at neutral pH. When bound to IgG at acidic pH, the receptor carries the immunoglobulin through the endocytic pathway in a way that avoids entry into the late endosome and lysosome. Instead, FcRn recycles the IgG back to the cell surface for release into the circulation at neutral pH, resulting in its long half-life.

The physiologic significance of the IgG-FcRn interaction has been recognized not only by immunologists but also by biotechnologists. In particular, the latter have appreciated that it can be exploited to enhance the half-life of therapeutic molecules linked to IgG or to deliver them across epithelial barriers via FcRn-dependent transport<sup>4,5</sup>. Detailed structural knowledge about the FcRn-IgG interaction<sup>3</sup> has allowed the generation of 'super IgGs' with increased affinity to FcRn and improved pharmacokinetics<sup>4</sup>.

There is a wrinkle, however, in this simple picture. Despite all their benefits, IgGs can also have pathogenic properties, as seen in various autoimmune conditions such as myasthenia gravis, immune thrombocytopenic purpura, Guillain-Barre syndrome and rheumatoid arthritis. Here, FcRn can be a therapeutic target. For example, blocking FcRn function in rodents, through deletion of the genes associated with FcRn expression, FcRn<sup>6</sup> and  $\beta_2$ -microglobulin<sup>7</sup>, or in humans, through administration of high doses of pooled immunoglobulins intravenously (IVIg)<sup>8</sup>, which presumably compete with endogenous IgGs, is effective in animal models of autoimmunity and in related diseases in humans. Such findings draw attention to the concept of inhibiting FcRn function to enhance degradation of IgG.

The relationships between FcRn, IgG and autoimmunity have not been lost on Vaccaro *et al.* Their new work builds upon substantial existing knowledge about the contact sites between FcRn and IgG, which influence IgG behavior in serum and the entire host. IgG binds FcRn through several critical amino acids in IgG that include Ile253, His310 and His435 (2,3). Vaccaro *et al.* modified human IgG in this region to create a mutated IgG that exhibits a 25-fold or higher increased



**Figure 1** FcRn normally protects IgG from degradation by diverting endogenous IgG from lysosomes. (a) It does this by binding IgG at the acidic pH (pH < 6.0) of endosomes after fluid phase endocytosis of IgG and recycling the bound IgG back to the cell surface where it is released at the neutral pH (pH > 7.0) of the interstitium (b). Antibodies that have been engineered to have increased binding at acidic pH but retain low binding at neutral pH exhibit a greater physiologic affinity for FcRn and thus have a longer half-life in serum and improved pharmacokinetics (c). "Abdegs" are antibodies that have been engineered to have increased nonphysiologic affinities for FcRn at neutral and acidic pH and therefore bind and do not readily release from FcRn. This blocks the protective function of FcRn for endogenous IgGs thus driving these to a degradative fate in lysosomes.

affinity for human (h) and mouse (m) FcRn at pH 6.0. Perhaps most importantly, the mutated IgG shows persistent and significant binding to hFcRn and mFcRn at pH 7.0, which, although reduced relative to binding at pH 6.0, is still high compared to that of wild-type IgG1.

The authors predicted that these properties of the mutated IgG, designated MST-HN, would 'clog' the protection machinery for endogenous IgGs, reducing their half-life. This idea was anticipated by earlier studies from the same group, which found that mutant IgGs with improved pharmacokinetic properties due to a slow off-rate at pH 7.0 and higher binding affinity at pH 6.0 were actually cleared more rapidly when injected into mice<sup>9</sup>.

Consistent with these predictions, MST-HN, when introduced into an FcRn-bearing cell *in vitro* or into a mouse together with a tagged wild-type IgG, forced accumulation of wild-type IgG into lysosomes and their rapid degradation, leading to a net decrease in endogenous IgG levels. Thus, Vaccaro *et al.* have created a new class of engineered antibodies, which they call 'Abdegs,' that enhance degradation of endogenous IgG.

Taken together with previous work by this group and others, this study shows that manipulation of amino acid residues around

Ile253, His310 and/or His435 can be used to generate antibodies (or antibody fragments) that bind FcRn with increased affinity at acidic pH but that still release the receptor at neutral pH (Fig. 1). Such antibodies or Fc-fusion molecules could be useful because of their enhanced pharmacokinetics (longer half-life)<sup>5,10</sup>. Alternatively, molecular manipulation of these same regions can produce an Fc-domain that binds FcRn with increased affinity independently of pH (as in Abdegs). These mutated antibodies do not release FcRn when they should, a property that might be useful for treating autoimmune diseases characterized by high concentrations of unwanted antibodies.

The potential utility of these findings, as noted by the authors, is quite significant. Currently, the treatment of antibody-mediated diseases is mainly limited to intravenous administration of exogenous IgG in very high concentrations. The injected antibodies enhance degradation of endogenous IgG by competing for binding to FcRn and inhibit B-cell production of IgG by binding classic Fc $\gamma$ -receptors<sup>8,11</sup>. The problem with this approach, however, is that IVIg is inefficient, unpredictable in its therapeutic benefit and decreases pathogenic antibodies, on average, by only 25–30% despite the enormous doses administered, which rival the total quantity of IgG present in a normal human.

Depletion of B cells is another therapeutic option; however, it removes not only IgGs produced by B cells but also many other potentially beneficial regulatory processes of these cells. A third approach, inhibition of specific effector functions of IgGs, such as complement activity, does not necessarily block all pathogenic properties of these immunologic structures.

In this context, Abdegs and their progeny may prove very useful for the treatment of antibody-mediated diseases. Related approaches include the development of antibodies that specifically neutralize FcRn, peptide mimetics that bind in critical regions of the FcRn-IgG interface or even small molecules that block FcRn function. Some support exists for the development of FcRn-blocking antibodies<sup>12</sup>, which are appealing given that they may bind FcRn with 100-fold higher affinity compared with Abdegs, which bind via the Fc-domain. Mimetics and small molecules may attain even higher affinities than those achievable with antibodies. In addition, the low molecular masses of mimetics and small molecules might allow administration of much larger molar concentrations of these inhibitors. These considerations are especially important as it is unknown whether Abdegs, or any other FcRn-targeted molecule, will function best below or above the FcRn saturation point. This latter point is quite important and deserves some additional comment.

FcRn is a saturable receptor, an insight attributed to F.W. Rogers Brandell<sup>2</sup>. Its

ability to regulate IgG metabolism is limited to the host's FcRn content, and it is estimated to be saturated at 10 mg/ml of IgG in the mouse and 35 mg/ml in the human. Humans with systemic lupus erythematosus often have greater than 40 mg/ml IgG in their serum<sup>6,13</sup>. An advantage of Abdegs may therefore be that, in contrast to IVIG, the increased affinities of Abdegs may allow efficacy at IgG concentrations that are well beyond the saturation point of FcRn. In the end, of course, all approaches to modulating FcRn function must be tested in humans. But the work of Vaccaro *et al.* represents an important start for this new direction in antibody engineering and the potential treatment of antibody-mediated diseases.

1. Vaccaro, C. *et al. Nat. Biotechnol.* **23**, 1283–1288 (2005).
2. Junghans, R.P. *Immunol. Res.* **16**, 29–57 (1997).
3. Martin, W.L., West, A.P. Jr., Gan, L. & Bjorkman, P.J. *Molec. Cell.* **7**, 867–877 (2001).
4. Bitonti, A.J. *et al. Proc. Natl. Acad. Sci. USA* **101**, 9763–9768 (2004).
5. Hinton, P.R. *et al. J. Biol. Chem.* **279**, 6213–6216 (2004).
6. Akilesh, S. *et al. J. Clin. Invest.* **113**, 1328–1333 (2004).
7. Liu, Z. *et al. J. Exp. Med.* **186**, 777–783 (1997).
8. Jin, F. & Balthasar, J.P. *Hum. Immunol.* **66**, 403–410 (2005).
9. Dall'Acqua, W.F. *et al. J. Immunol.* **169**, 5171–5180 (2002).
10. Ghetie, V. *et al. Nat. Biotechnol.* **15**, 637–640 (1997).
11. Samuelsson, A., Towers, T.L. & Ravetch, J.V. *Science* **291**, 484–486 (2001).
12. Getman, K.E. & Balthasar, J.P. *J. Pharm. Sci.* **94**, 718–729 (2005).
13. Roopenian, D.C. *et al. J. Immunol.* **170**, 3528–3533 (2003).

rather than of design. This 'black art' reputation has discouraged ion-channel biologists from attempting to generate more of these useful tools. Where does one begin in designing an inhibitory antibody against a typical channel sequence of >1,000 amino acids? The first and most obvious requirement is an antibody recognition site that is extracellular and hence accessible in a physiological environment.

The largest superfamily of ion channels includes the voltage-gated Na, Ca and K channels as well as the transient receptor potential (TRP) Ca channels (Fig. 1). These six-transmembrane-domain channels are typified by the voltage-gated K channels. Hydrophobicity analysis and mutagenesis studies indicate that their six transmembrane helices are connected by extracellular and intracellular loops, a picture that has been elegantly verified by X-ray crystallography<sup>3</sup>. The pore through which K ions flow is made up of two transmembrane helices and a loop in the extracellular linker that dips back into the membrane from each of four copies of the protein. In contrast to voltage-gated K channels, voltage-gated Na and Ca channels come preassembled: they comprise four subunits in a single polypeptide sequence rather than four proteins in a complex.

Using this basic template, we can make a reasonable prediction for any six-transmembrane-domain channel as to which amino acids are exposed on the extracellular surface in the loops between helices S1 and S2, S3 and S4 and in the more complex loop between S5 and S6 (named E1, E2 and E3, respectively, by Beech and colleagues) (Fig. 1). Fortunately, although the transmembrane helices generally show high sequence similarity across ion channel families, the connecting loops have considerable sequence diversity, providing an opportunity to generate specific antibodies. But this requirement doesn't further narrow the choice of extracellular exposed sequence.

Beech and his colleagues have been cooking up antibodies for a number of years to elucidate the functions of K and TRP channels in vascular smooth muscle cells<sup>4</sup>. They need tools that are useful in isolated, native tissue samples. Antibodies admirably fit this bill.

In analyzing previous limited successes with four inhibitory antibodies against two K channels and a TRP channel (TRPC1)<sup>5–7</sup>, Beech and colleagues found that the antibodies were invariably targeted to the channels' E3 region, that is, the amino acid sequence C-terminal to transmembrane helix S5, just before the structure dips back into the membrane in the pore loop that contributes to pore structure (Fig. 1). Could antibodies against E3 sequences represent a general

## Simple recipe for blocking ion channels

Christopher D Benham

**Ion channels can be inhibited by polyclonal antibodies targeted to a specific extracellular loop.**

Genome sequencing has provided electrophysiologists with a plethora of new ion channel sequences. Not surprisingly, the supply of pharmacological reagents to probe their

functions has lagged behind. In this issue, Beech and colleagues<sup>1</sup> offer a simple and elegant recipe for producing specific 'inhibitory' polyclonal antibodies that can be targeted to any ion channel with a six-transmembrane-domain structure. Such antibodies will facilitate the study of excitable cells and, as many of these channels are potential drug targets, may provide a starting point for the development of therapeutic antibodies.

Most existing antibodies against ion channels do not perturb channel function<sup>2</sup>. Those that do are largely the result of serendipity

Christopher D. Benham is at the Neurology and GI Center for Excellence in Drug Discovery (N&GI CEDD), GlaxoSmithKline Research and Development Ltd., New Frontiers Science Park (North), Harlow, Essex, CM19 5AW, UK. e-mail: christopher.d.benham@gsk.com