RESEARCH NEWS

Antibodies live long and prosper

Lutz Riechmann and Philipp Holliger

The success of immunoglobulins or immunoglobulin fragments in disease treatment is dependent on many variables. Protein engineering is being increasingly used to modify and improve those characteristics. Much progress has been made recently in obtaining, to order, antigen-binding sites with high affinity and specificity using phage display libraries[1], as well as in engineering novel effector functions, for example, through the recruitment of cytotoxic T cells[2]. Thus far, a property that has been largely untouched by antibody engineering is the serum persistence of immunoglobulins in the patient. Now, Ghetie et al.[3] report in this issue the design of a recombinant murine immunoglobulin fragment with a significantly increased serum half-life in mice. They achieved this by enhancing the binding affinity of the immunoglobulin fragment for the neonatal Fc receptor (FcRn).

A prolonged half-life is desirable in therapy for many reasons. In particular, it should render immunotherapeutic approaches more effective. In addition, the need for less-frequent administration should make such reagents cheaper and more accessible to clinicians.

The serum persistence of an administered antibody is governed by two overlapping processes. Initially, a very fast drop in serum concentration of antibodies occurs (the α phase) as a result of extravasation, reaching an equilibrium during the first day after administration. After this fast initial drop, the natural catabolism of the antibody is mostly responsible for its disappearance from the serum (β phase). Compared with other serum proteins of similar size, IgG has a much extended residence time in the serum and, as early as 1964, a salvage receptor specific for IgG was proposed[4] that could capture IgG inside endosomes and recycle it to the serum.

Lutz Riechmann and Philipp Holliger are research scientists at the MRC Laboratory of Molecular Biology and MRC Centre for Protein Engineering, Hills Road, Cambridge CB2 2QH, UK (lutz@mrc-lmb.cam.ac.uk).

Recently, the salvage receptor has been identified as the neonatal Fc receptor[5], which was initially identified based on its ability to transport intact IgG across the neonatal placenta[6].

Binding of IgG to FcRn shows a sharp pH dependence: It is strong at acidic pH and weak at neutral pH. IgG is therefore tightly bound after pinocytosis and acidification in the endosome, where binding to FcRn reroutes IgG away from the lysosomal pathway back to the cell surface. There, it is re-released in the neutral pH of the extracellular fluid (Fig. 1). The acidic environment of the endosome usually also purges the IgG of bound antigen, which can then be degraded in the lysosome.

Although three other FcR receptors are specific for IgG[7] (FcγRI, FcγRII, and FcγRIII), none of these appears to influence IgG serum homeostasis[5,8]; rather, they trigger IgG effector functions. The FcγR binding sites do not overlap with that for FcRn. The approach of Ghetie et al.—using site-directed random mutagenesis and phage display of an IgG Fc fragment to enhance the affinity for FcRn at pH 6—should therefore not affect the ability of the IgG to recruit immune effector functions. Remarkably, even the pH dependence of binding to FcRn remained largely intact, even though the dissociation rate at pH 7.4 was slowed down. However, the increase achieved in serum half-life (from 120 to 150 hours in mice) is not directly proportional to the affinity increase (threefold). This may indicate that a slower off-rate at pH 7.4 can partly offset the advantage of a tighter binding at pH 6.

This success should initiate other attempts to create even better FcRn binding sites on the IgG molecule. It also suggests strategies to enhance the short half-life of smaller antibody fragments devoid of Fc portions. Thus, clearance could be slowed by direct binding to FcRn. Antibody fragments or peptides with an affinity for FcRn at low pH could be designed or selected and used as tags to result in catabolic salvage of the fusion partner. Binding of such tags to FcRn should also be weaker at neutral pH to allow efficient dissociation during recirculation. The design and selection of such behavior may well be challenging, in particular for short peptides.

Alternatively, protection of smaller proteins through FcRn conferred salvage might be achieved by acid-stable binding to IgG (i.e., FcRn binding by proxy). This strategy has the advantage of additionally transferring antibody effector functions to the reagent. The approach has already been partly realized using a bispecific antibody fragment (diabody) with one antigen-binding site dedicated to bind a conserved epitope on serum IgG[9]. However, as the IgG binding by the diabody is presumably acid-labile, the maximum serum half-life achieved so far seems to be limited to that of IgG in mice devoid of FcRn[5]. Even so, the selection of acid-stable IgG binders using phage-technology should be relatively straightforward.

In the future, we expect a range of measures to be available to finetune the pharmacokinetics of antibodies, antibody fragments and perhaps also other recombinant proteins for both imaging and therapy. The work of Ghetie et al. is surely just the beginning.

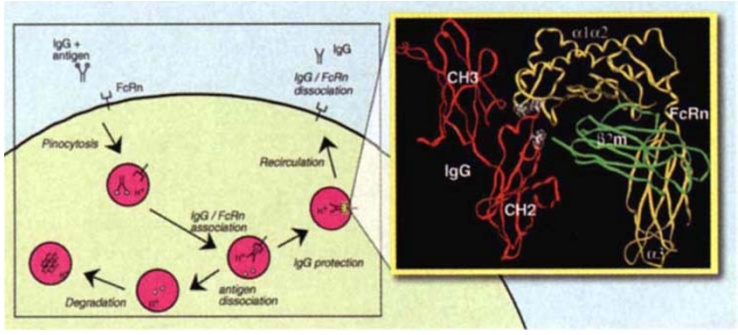

Figure 1. (A) The FcRn salvage receptor protects IgG from catabolic degradation through tight binding in the acidic endosomes and recircluates them into the neutral, extracellular environment after dissociation (Figure adapted from ref 5). (B) The three IgG residues mutated by Ghetie et al.[3] to improve FcRn binding are central to the interface of the FcRn-IgG complex[10].

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