Pharmaceutical BIOPROCESSING

Antibody immunogenicity: does bioprocessing hold all the answers?

"...antibody therapy with engineered products has provided a wealth of useful 'billion dollar' drugs."

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Herman Waldmann
Sir William Dunn School of Pathology, South Parks Road, Oxford, OX1 3RE, UK
herman.waldmann@path.ox.ac.uk

Monoclonal antibodies offer major opportunities for therapy of chronic inflammatory diseases and cancer. Molecular engineering has been adopted to ensure that they can be rendered human-like with the hope of eliminating immunogenicity. This aspiration has not, though, been realized for many antibodies, and for all patients. Although improvements in bioprocessing can minimize immunogenicity arising from unwanted protein aggregates, the intrinsic nature of each antibody, its target and its mode of action can also impact their capacity to generate neutralizing host antibody responses. Exploitation of the body's immune tolerance mechanisms could, in principle, overcome these remaining limitations.

The therapeutic potential of monoclonal antibodies (Mabs) became widely appreciated when their human equivalents could be engineered. These engineered products could be tailored to fulfill desired effector functions, and the hope was that rendering them 'human' [1–3] would prevent them evoking neutralizing immune responses in the host. Consequently, antibody therapy with engineered products has provided a wealth of useful 'billion dollar' drugs. Contrary to expectations though, the creation of 'human-like' antibodies has not completely eliminated immunogenicity [4]. Although attention to methods of bioprocessing can minimize creation of immunogenic post-translational products and aggregates, there are intrinsic features of some antibodies that endow them with adjuvanticity in generating neutralizing host responses.

We have long known that 'foreign' immunoglobulins, just like other foreign proteins, can evoke immune responses in humans and in experimental animals, Human immunoglobulins, 'foreign' to mice, were able to induce antibody responses in mice when artificially aggregated by heating, but did not do so when given as IgG monomers. Instead, immunoglobulin monomers could act as tolerogens in injected hosts [5]. At high doses monomers tolerized both antigen-specific T-helper cells and B-cells, and at low doses tolerized the T-helper cells only. In short, tolerization of T-helper cells by monomers was sufficient, in mice, to prevent antibody responses even to immunogenic foreign aggregates. This 'classic' finding provides us with a valuable basis for strategies to avoid neutralizing anti-drug responses, as will be discussed later.

Many therapeutic Mabs have been used to target antigens on easily accessible cells in the blood and lymphoid systems. Binding of many antibody molecules to the surface antigens of individual cells, can, in principle, create a more 'physiological' type of 'immunogenic' aggregate. Moreover, adjuvanticity can be generated by the secondary destructive and inflammatory events which follow antibody binding [6]. This we demonstrated in 1986, when we observed that many rat antibodies directed toward murine leucocyte antigens were immunogenic, while other rat Mabs that were non-binders did not produce antibody responses. Instead, they were able to tolerize mice to later challenge with aggregates [6].

It is not just cell–surface binding that matters. Some therapeutic Mabs may target soluble antigens that are multimeric (e.g., TNF family members) and consequently generate

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immunogenic complexes that also elicit adjuvanticity from the inflammatory or 'danger' signals they create. Adjuvanticity might also be enhanced through agonist activity of some of these multimeric receptors.

Avoidance of immunogenicity from such aggregates generated within the patient therefore requires interventions additional to just humanization.

First, we might identify and eliminate all remaining foreign T-helper cell epitopes (ones to which the host has not become naturally tolerant) [7,8]. Such epitopes would be those peptide sequences within the protein that could sit in the pocket of MHC Class II molecules involved in their presentation, and could be recognized as foreign by the T-cell receptors of host helper T-cells. 'Deimmunization' of a therapeutic antibody might involve, for example, the elimination of T-helper cell epitopes by mutagenesis. Although widely accepted as a principle, there are still few, if any, clinically proven examples of therapeutic antibodies rendered silent through this route.

Second, we might aim to induce tolerance to the culpable T-helper cell epitopes using principles based on the creation of T-cell 'helplessness' of the kind observed by Chiller and Weigle [5]. To achieve this, we developed a novel strategy, one that could be used to supplement humanization [9,10].

Humans are tolerant of the constant or framework regions of their own antibodies through processes encompassed in the term 'self-tolerance.' Humanization or the derivation of so-called 'fully human' monoclonal antibodies generates products with complimentarity determining regions (CDRs) to which patients would not have acquired helper-T-cell tolerance, either to the CDR elements themselves, or to peptide sequences which overlap the CDRs and their adjacent supporting framework sequences. For example, the humanized CD52 antibody (CAMPATH-1H or alemtuzumab) elicited strong anti-idiotypic responses in the majority of patients treated [11]. This is evidence that the CDRs do remain a focus for host immunity towards 'human' therapeutic antibodies.

Our tolerizing strategy aims to tolerize the T-helper cells (those that would otherwise be used to generate antibody responses), to the residual 'foreign,' epitopes involving the CDRs. We speculated that non-cell-binding and tolerogenic forms of the therapeutic antibody might be generated through creating limited number of mutations in CDRs critical to antigen binding [10]. Such non-binding variants given ahead of the therapeutic form might, we argued, induce tolerance to the therapeutic form.

The feasibility of this approach was shown in mice transgenic for human CD52 expressed on their white blood cells. Mutant non-binding forms of the humanized CAMPATH-1H antibody given to these animals did not elicit antibody responses. Booster challenges with either the mutant form, or even with the therapeutic form, also failed to evoke responses [10]. This demonstrated that, just as predicted from the Chiller and Weigle experiments, monomeric non-binding immunoglobulins can tolerize to the binding, otherwise, immunogenic, forms.

A similar outcome was observed in a small-scale clinical study using such a two-stage approach. A high dose of mutant non-binding version of CAMPATH-1H (Alemtuzumab), given before treatment with the therapeutic version, substantially reduced primary and secondary antibody responses to the therapeutic non-mutated form [11].

Given this finding, one might ask why such a strategy has not been adopted by the pharmaceutical industry? The likely reason is the major disadvantage that two pharmaceutical products, the tolerogen and the therapeutic, are required – and the logistics of commercial development of such a package are, unfortunately, forbidding.

It is, though, not beyond the stretch of the imagination that one could produce a single therapeutic product that could serve both as a tolerogen and as a therapeutic. These functions could be temporally separated. This would require that the bulk of the antibody be non-binding (tolerogenic) in the first few days after administration, after which the therapeutic antibody would acquire access to its target, at a later stage, once tolerance has been induced. We have exemplified the utility of such a one-step strategy using a blocking mimotope covalently inserted into the antigen-binding-site of an antibody [12]. Despite evidence for its utility, this approach has yet to attract the interest of drug developers. Maybe it is just a question of time, or maybe there is a sense of denial of the problem that needs to be dealt with. Perhaps more likely, it is the thought that once a given antibody is neutralized by the host response, other antibodies with different sequences can be rotated in to replace them. This seems to have been the route, thus far, for drugs targeting TNF in rheumatoid arthritis.

What about antibodies that target antigens outside the blood and lymph systems? It may, fortuitously, be the case, that Mabs to such targets, have less of an immunogenicity problem. Administration of the drug intravenously immediately creates an equilibrium with the bulk of 'unbound' antibody in the blood stream. For a while this would remain in large excess over that equilibrating with the tissues. Given that, there may be conditions (dose and antigen location) where the race between tolerization and immunization would favor the former, especially at high antibody doses.

In summary, there may be limits to what can be done to reduce Mab immunogenicity by simple engineering and bioprocessing approaches. Relatively simple

tolerization strategies could be applied to complete the job, were there the will to overcome the logistic obstacles.

Financial & competing interests disclosure

H Waldmann is one of the authors of US patent 7465790 B2 mentioned in the text. The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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