

Antidotes, antibody-mediated immunity and the future of pharmaceutical product development

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If new scientific knowledge is to be more efficiently generated and applied toward the advancement of health, human safety must be more effectively addressed in the conduct of research. Given the present difficulties of accurately predicting biological outcomes of novel interventions *in vivo*, the imperative of human safety suggests the development of novel pharmaceutical products in tandem with their prospective antidotes in anticipation of possible adverse events, to render the risks of initial clinical trials more acceptable from a regulatory standpoint. Antibody-mediated immunity provides a generally applicable mechanistic basis for developing antidotes to both biologicals and small-molecule drugs (such that antibodies may serve as antidotes to pharmaceutical agents as a class including other antibodies) and also for the control and prevention of both infectious and non-infectious diseases via passive or active immunization. Accordingly, the development of prophylactic or therapeutic passive-immunization strategies using antipeptide antibodies is a plausible prelude to the development of corresponding active-immunization strategies using peptide-based vaccines. In line with this scheme, global proliferation of antibody- and vaccine-production technologies, especially those that obviate dependence on the cold chain for storage and transport of finished products, could provide geographically distributed breakout capability against emerging and future health challenges.

Keywords: human safety, pharmaceutical product development, drugs, biologicals, antidotes, antibody-mediated immunity, antipeptide antibodies, peptide-based vaccines

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Introduction

The genomic revolution raised expectations of unprecedented advances in health care,^{1,2} but actual scientific progress remains constrained by the double bind of limited available empirical data and concerns over human safety in research. Failure to address this issue could result in general disappointment over inadequate translation of scientific knowledge into actual improvements in the quality of human life, leading to loss of confidence in science-based initiatives and continued trends toward pseudoscientific and antiscientific alternatives epitomized by the antivaccination movement.³ On a more positive note, timely resolution of the issue could empower the global health system. This commentary thus aims to outline possible future directions of health research with emphasis on antibody-mediated immunity as a concept central to pharmaceutical product development that prioritizes human safety.

Global Health and Translational Science

Global health may be defined as “collaborative transnational research and action for promoting health for all,” wherein “health for all” refers back to the 1978 Declaration of Alma Ata as proclaimed by the World Health Organization (WHO).⁴ In the Preamble to the WHO Constitution, health itself is defined as “a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity,” which has been criticized as practically meaningless.⁵

A more pragmatic view of health is that of just health, which entails meeting health needs fairly via consensus-building among stakeholders, subject to real-world constraints on available resources.⁶

To inform the negotiation of just health, health outcomes must be predicted for resource-allocation options. Modern science, in the sense of empirically validated predictive mathematical models,^{7,8} provides means for predicting outcomes relevant to health and other human needs, thereby enabling translation of scientific knowledge into practical applications.⁹ This motivates the pursuit of biomedical research, often with the expectation that reductionist approaches will continue to drive the generation of new knowledge.¹⁰ Undeniably, reductionism has enabled science-driven technological revolutions, but negative unintended consequences of technological change caution against overly simplistic reductionist approaches.¹¹ Global health is thus conditioned by the bioethical principle of nonmaleficence (i.e., avoidance of causing harm), which is subsumed under the precautionary principle (i.e., assigning the burden of proof to proponents of activities that may threaten health and the environment).¹²

Such risk aversion is reflected in prevailing regulatory regimes, which remain committed to using animal models for evaluating safety under the assumption that such models are valid for human biomedical applications despite interspecies biological differences.¹³ Meanwhile, animal-welfare concerns present a growing barrier to animal use, especially with the validity of animal models being called into question.¹⁴ Hence, translational science must meet demands for evidence of safety while being denied conventional means for producing such evidence.

Animal-based safety studies might be supplanted by Phase 0 clinical trials, whereby pharmacokinetic and pharmacodynamic properties of investigational new drugs are initially explored using subtherapeutic doses administered to healthy human volunteers.^{15,16} Still, even seemingly low doses may produce adverse effects in ways that are difficult to predict for new drugs by virtue of their novelty.

Complexity, Uncertainty and Acceptable Risk

Biological complexity limits the applicability of reductionism framed with deterministic linear mathematical models,⁸ which historically have dominated science as they are more computationally tractable and humanly comprehensible than stochastic nonlinear mathematical models more appropriate for describing and predicting biological systems behavior. Such stochastic models must be developed and validated on the basis of empirical data if they are to be useful for health-care decision support.⁷ Unfortunately, the required data are still critically lacking; and even if this paucity of data were overcome, uncertainty would still be inherent in the predictions generated by any stochastic models developed. Reluctance to permit initial trials of novel interventions on the grounds that they entail unacceptably uncertain risk levels itself limits the generation of new empirical data that might yield better estimates of the said risk levels, thereby perpetuating a vicious circle of risk aversion and limited capacity for risk assessment.

To break the aforementioned vicious circle, one possibility is to develop new interventions in conjunction with specific countermeasures from the outset, in the interest of safety. Where the interventions would entail the administration of pharmaceutical agents, the countermeasures could include the administration of specific antidotes, such that new pharmaceutical agents would be concomitantly developed in tandem with their antidotes.

Agents, Antidotes and Antibody-Mediated Immunity

Given a novel candidate pharmaceutical agent, a corresponding prospective antidote might be developed on the basis of specific ligand-receptor interactions. If the agent is regarded as a ligand that binds to a particular target receptor, the antidote might be an alternative ligand that competitively binds to the same receptor; otherwise, the antidote might be an alternative receptor that competes with the target receptor for binding by the

agent. Developing antidotes as alternative ligands necessitates sufficiently detailed knowledge of the target receptors, which nonetheless fails to guarantee production of alternative ligands that actually function as antidotes (e.g., as pharmacologic antagonists rather than pharmacologic agonists). In contrast, developing antidotes as alternative receptors is amenable to implementation by means of a generally applicable strategy, namely the production of specific antibodies against virtually any agent, such that antidote effects are realized as antibody-mediated immunity.

The production of antibodies against pharmaceutical agents is well-known for biologicals, which tend to be immunogenic. This is problematic where biologicals elicit antibody responses that compromise efficacy and result in hypersensitivity exemplified by serum sickness, wherein a systemic hypersensitivity reaction develops against heterologous serum proteins, as may occur with serum therapy (i.e., passive immunization using serum antibodies). Serum therapy was extensively employed in the treatment of infectious diseases prior to the widespread availability of antibiotics¹⁷ and is a classic example of antibody-based prophylactic and therapeutic approaches, for which antibodies of human origin or having humanized sequences tend to be preferred over unmodified nonhuman antibodies in contemporary clinical practice.¹⁸

A more recent example of antibody-mediated immunity to biologicals is that of neutralizing autoantibodies against therapeutic erythropoietin, which tend to be produced as a result of pharmaceutical product adjuvanticity due to suboptimal manufacturing processes.¹⁹ Such problems can occur where proteins in biologicals contain potentially immunogenic T-cell epitopes as is typical even among autologous proteins, which has motivated efforts to minimize the immunogenicity of biologicals via T-cell epitope deletion.²⁰ Yet, the immunogenicity of even autologous proteins suggests the possibility of producing functionally neutralizing antibodies against virtually any self-protein. Neutralizing anticytokine antibodies have thus been developed for the treatment of inflammatory conditions wherein excessive cytokine production contributes to

immunopathology;²¹ and such antibodies may also serve as antidotes to therapeutic cytokines, for mitigating cytokine-mediated toxicity.

Additionally, antibodies can also serve as antidotes to small-molecule drugs. Although such drugs are typically haptens, antibodies may nonetheless be produced against them (e.g., by effectively rendering them immunogenic via conjugation to strongly immunogenic carrier molecules). This enables, for example, the production of antibodies against the cardiac glycoside digoxin, which yield Fab fragments useful as an antidote for toxicity due to digoxin and even other structurally similar cardiac glycosides.^{22,23}

To place the role of antibodies as antidotes into proper perspective, this is but only one aspect of antibody-mediated immunity, which encompasses a rich variety of immune effector mechanisms that can potentially contribute to the control and prevention of clinical conditions in general.²⁴ Whereas such immunity was previously thought of as being confined to extracellular spaces, effector mechanisms have been identified that operate within intracellular spaces as well, for example, within endosomes (e.g., of epithelial cells wherein internalized dimeric IgA complexed with the polymeric-immunoglobulin receptor pIgR mediates intracellular neutralization of invading virions²⁵) and even cytoplasm (e.g., of cells expressing the ubiquitin ligase TRIM21, which catalyzes the ubiquitinylation of IgG-bound viral capsids to facilitate their proteasomal degradation and thereby interrupt the viral replication cycle²⁶). Antibody-mediated immunity can contribute to the control of both infectious and noninfectious diseases, in the latter case possibly by selective targeting of self molecules. However, antibody-mediated immunity itself may contribute to adverse effects, including antibody-mediated enhancement of infection (as observed among various viral and cellular pathogens,²⁷ especially enveloped viruses in settings of low-affinity antibody binding and low antibody concentration) and excessive loss of self-molecule function.

From the standpoint of safety, passive immunization by administering exogenous antibodies is preferable to active

immunization that induces endogenous antibody production, considering the potential complications of active immunization in the event of adverse antibody-mediated effects. Although such effects could be mitigated in either passive or active immunization schemes by administering an antidote (e.g., an antibody that binds and thereby neutralizes the antibody mediating adverse effects), passively acquired antibodies eventually would be eliminated from the recipient, in which case administration of the antidote might be unnecessary if antibody elimination kinetics are sufficiently rapid; but active immunization may induce protracted endogenous production of antibodies, such that the antidote might be administered repeatedly over a prolonged and possibly indefinite period. Furthermore, in the absence of any available antidote, the adverse effects of active immunization might be essentially permanent, which argues for the development of antidotes for such effects where active immunization is contemplated.

The preceding considerations, particularly the potential benefits of developing antidotes to prophylactic and therapeutic agents, suggest a general strategy for pharmaceutical product development based on monoclonal antibodies. Structurally distinct monoclonal antibodies may be generated against a pharmaceutical agent, such that each monoclonal antibody may serve as a distinct alternative antidote to the agent, thereby providing a range of possible antidotes from which may be selected one that is most appropriate for administration to a particular patient (e.g., to avoid idiosyncratic hypersensitivity reactions).

Nevertheless, the historical cost-effectiveness of antibody-mediated immunity in public-health terms has been overwhelmingly due to active immunization, via mass vaccination, rather than passive immunization. Although novel prophylactic and therapeutic approaches based on antibody-mediated immunity are likely to be more acceptable in terms of safety if initially realized via passive rather than active immunization, economic considerations would motivate subsequent attempts to induce similar immunity via active immunization (e.g., by vaccination),

which would entail polyclonal rather than monoclonal antibody responses. This poses the problem of developing vaccines that are efficacious yet safe, particularly in the sense of also developing antidotes that mitigate antibody-mediated adverse effects that might occur consequent to active immunization with the vaccines.

Antipeptide Antibody-Mediated Immunity and Peptide-Based Vaccines

B-cell epitope specificities of monoclonal antibodies produced against the same immunogen may vary widely even among clones from the same immunized individual. Although investigators may selectively retain clones with desirable properties (e.g., secretion of protective antibodies) while discarding other clones with undesirable properties (e.g., secretion of non-protective antibodies that actually enhance infection), such artificial selection is much less feasible *in vivo*. This presents a major challenge when attempting to develop active-immunization schemes (e.g., vaccination) that recapitulate antibody-mediated immunity achieved via passive immunization with monoclonal antibodies. If, for example, a protein contains multiple structurally distinct B-cell epitopes, *in-vivo* polyclonal antibody responses to the protein are likely to yield antibodies that target the different epitopes, albeit to varying extents as a function of multiple factors including immunogen structure (which may be at least partially denatured in the course of immunization²⁸), host genetic background (e.g., favoring tolerance toward epitopes similar to those of self antigens) and history of prior immunization (e.g., resulting in original antigenic sin,²⁹ wherein subsequent immune responses are biased toward epitopes resembling previously encountered ones); at worst, the immune responses can be completely non-protective (e.g., by failing to produce protective antibodies against key target epitopes) and also harmful (e.g., by producing antibodies that mediate enhancement of infection).

To address the problem of developing active-immunization schemes for optimal targeting of polyclonal antibody

responses against polyepitopic targets, artificial means may be employed to avoid counterproductive immunodominance (i.e., to artificially bias immune responses toward protective epitopes and away from other epitopes that may be non-protective and even disease-enhancing). One proposed approach is immune refocusing,³⁰ whereby immune responses are selectively focused on intended target epitopes (e.g., of pathogen-neutralizing antibodies) on engineered variants of naturally-occurring macromolecular targets from which other potentially immunodominant epitopes (e.g., immunological decoys evolved by pathogens to evade host immune systems) have been deleted; however, this is technically challenging insofar as the selective epitope deletion should maintain the intended target epitopes intact. An alternative approach is the use of peptide-based immunogens to elicit antipeptide antibodies that cross-react with functionally critical epitopes on the intended macromolecular targets.³¹

The use of antipeptide antibodies to produce intended biological effects is straightforward where the actual target is itself a peptide, especially where it is so short as to comprise only one or very few B-cell epitopes, for example, in the case of the octapeptide angiotensin II.³² In such cases, the immunizing peptide may structurally mimic the target peptide such that the elicited antipeptide antibodies cross-react with the target peptide. A major challenge is posed by the problem of ensuring that antipeptide antibodies actually cross-react with protein targets; even where the immunizing-peptide sequence is chosen to exactly match a segment of a target protein with which the antipeptide antibodies thus obtained are intended to cross-react, cross-reaction may fail to occur (e.g., because the corresponding protein sequence is structurally inaccessible or in a conformation different from that of the immunizing peptide). Moreover, where cross-reaction does actually occur, this fails to ensure that intended biological effects (e.g., protection against rather than enhancement of disease) are produced. These challenges historically have hindered progress toward developing peptide-based vaccines, in spite of extensive

computationally-guided work focused mainly on B-cell epitope prediction.³³

Nevertheless, the development of peptide-based immunogens as vaccines has become the subject of renewed interest in recent years, especially with improvements in computational support for vaccine design and in vaccine technology overall.^{34,35} With regard to B-cell epitope prediction for peptide-based immunogen design, the computational problem is now better understood in terms of physicochemical and biological correlates,^{36,37} and many new epitope-prediction tools are now available.³⁸ Furthermore, the realizations that antibody-mediated immunity may pose unacceptable risk of harm in certain cases (e.g., due to antibody-dependent enhancement of disease) and that purely cell-mediated immunity (i.e., based on adaptive T-cell immunity) may be effective in disease prevention suggest the prospect of peptide-based vaccines designed to include primarily (if not exclusively) T-cell epitopes.³⁹

Within the conceptual framework of pharmaceutical agents and antidotes that has been outlined thus far, risk aversion toward peptide-based vaccines could be mitigated by developing antibodies and peptides as antidotes. Beyond *in-silico* preliminary analyses to avoid introducing potentially harmful peptide-vaccine sequences (e.g., those similar to epitopes of self, food or common environmental components), candidate vaccine peptides could be incorporated into immunogens for producing antipeptide monoclonal antibodies as candidate therapeutic or prophylactic agents. Before administering these antibodies to humans, prospective antidotes to the antibodies could be developed for use in the event of any adverse antibody-mediated effects that might be observed among human subjects. The antidotes could be anti-idiotypic monoclonal antibodies (e.g., generated via immunization with the antipeptides antibodies or variable-region fragments thereof) or the peptides themselves (e.g., as unconjugated haptens in monomeric form to avoid proinflammatory immune-complex formation via antibody cross-linking), such that the anti-idiotypic antibodies would inhibit antigen binding by the antipeptide antibodies while the peptides would

directly block the antipeptide-antibody paratopes. Insofar as proteins and peptides are typically metabolized *in vivo* via main-chain peptide-bond hydrolysis,⁴⁰ human-safety concerns over the prospective antidotes would be less problematic than for more exotic xenobiotics whose *in-vivo* toxicity is much more difficult to predict (e.g., in view of potentially complex biotransformation pathways⁴¹). The prospective antidotes might be less costly to manufacture as peptides rather than anti-idiotypic antibodies considering that chemical synthesis of the peptides would circumvent resource-intensive antibody production, although regulators may favor novel antibodies over small-molecule new chemical entities.⁴²

Demonstrating the safety of antipeptide antibodies in humans may provide justification for further studies on active immunization with peptide-based immunogens, toward vaccine development. Efficacy of the antipeptide antibodies for therapeutic or prophylactic immunization could motivate further vaccine-development efforts, although such efforts might nonetheless be justified on the basis of cell-mediated immunity. In the event of any adverse effects due to endogenous antipeptide antibodies elicited by immunization with the peptide-based immunogens, the effects might be mitigated by administering peptides previously developed as antidotes to similar antibodies used for passive immunization.

Thus developing peptide-based vaccines as synthetic chemical products would conceivably entail lower manufacturing costs and fewer biosafety concerns relative to conventional vaccine-production technologies that utilize biological materials. In contrast to biologically derived proteins and more complex materials (e.g., containing viable pathogens or attenuated variants thereof), synthetic peptides are more amenable to nondestructive lyophilization for extension of product shelf life by avoiding solvent-mediated hydrolysis, such that a shift from conventional to peptide-based vaccines could obsolete the cold chain, which is an extremely crucial yet technically and economically demanding aspect of conventional vaccine logistics.⁴³ Peptide-based vaccines and, more generally, peptide-based immunogens

could also play an instrumental role in the production of polyclonal antipeptide antibodies as therapeutic and prophylactic agents, considering that such antibodies could be recovered from human sources (e.g., via processing of expired human-donor plasma from medical blood-product banking facilities) or even immunized animals (as has been practiced since the advent of serum therapy, noting that this still remains a cost-effective measure, e.g., for treating tetanus using equine antitetanus antiserum,⁴⁴ in the absence of contraindications such as known allergies or potential hypersensitivity reactions due to repeated dosing).

Conclusions

The development of new therapeutic and prophylactic pharmaceutical agents in tandem with their corresponding antidotes is a plausible generally applicable strategy to resolve the current impasse over human-safety concerns as regards pharmaceutical products. In this context, antibodies can potentially fulfill dual roles as both antidotes and pharmaceutical agents in their own right, possibly for passive-immunization approaches that may provide proof of concept for subsequent active-immunization approaches such as vaccination. Pharmaceutical antipeptide antibodies in particular can be targeted against individual epitopes, thereby facilitating evaluation of these epitopes from the perspectives of safety and efficacy, as a prelude to selective combination of epitopes in the design of peptide-based immunogens as vaccines. Ultimately, the proliferation of geographically dispersed low-cost manufacturing of peptide-based vaccines and of pharmaceutical antipeptide antibodies could better enable the global health system for high-capacity yet locally responsive health-product development and distribution, proceeding along paths that explicitly prioritize human safety in scientifically well-grounded and practically meaningful ways toward the goal of just health.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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