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MINIREVIEW

Antibody Pharmacokinetics and Pharmacodynamics

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ABSTRACT: The U.S. Food and Drug administration (FDA) has approved several polyclonal antibody preparations and at least 18 monoclonal antibody preparations (antibodies, antibody fragments, antibody fusion proteins, etc.). These drugs, which may be considered as a diverse group of therapeutic proteins, are associated with several interesting pharmacokinetic characteristics. Saturable binding with target antigen may influence antibody disposition, potentially leading to nonlinear distribution and elimination. Independent of antigen interaction, concentration-dependent elimination may be expected for IgG antibodies, due to the influence of the Brambell receptor, FcRn, which protects IgG from catabolism. Antibody administration may induce the development of an endogenous antibody response, which may alter the pharmacokinetics of the therapeutic antibody. Additionally, the pharmacodynamics of antibodies are also complex; these drugs may be used for a wide array of therapeutic applications, and effects may be achieved by a variety of mechanisms. This article provides an overview of many of the complexities associated with antibody pharmacokinetics and pharmacodynamics.
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INTRODUCTION

Interest in the development of antibody drugs is rapidly increasing and, over the last several years, more than 20 antibody products (i.e., antibodies, antibody fragments, or antibody-fusion proteins) have been approved by the U.S. Food and Drug Administration (FDA) (Tables 1 and 2). Additionally, there is increasing interest in the use of pharmacokinetic and pharmacodynamic analyses to guide and expedite drug development.[1] As such, it may be timely and instructive to review the pharmacokinetic and pharmacodynamic characteristics of antibodies. As discussed below, antibody drugs may achieve effects by a variety of mechanisms, and antibodies are often associated with nonlinear distribution and metabolism (Fig. 1); consequently, antibodies often exhibit pharmacokinetic and pharmacodynamic properties that are much more complex than those typically associated with small-molecule drugs. This review will provide the reader with an introduction to general issues associated with antibody structure (e.g., isotypes, fragments, etc.), antibody pharmacokinetics, and antibody pharmacodynamics.

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ANTIBODY STRUCTURE

In humans, antibodies may be classified as members of five families (or isotypes), which have been named immune globulin alpha (IgA), delta (IgD), epsilon (IgE), gamma (IgG), and mu (IgM).[2] Structural differences between the isotypes, summarized in Table 3, include differences in molecular weight (ranging from ~150 to ~1150 kDa)

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Table 1. U.S. Food and Drug Administration (FDA)-Approved Therapeutic Monoclonal Antibodies, Fragments, and Fusion Proteins

Name	Antibody Isotype	Binding Target	Apparent Volume of Distribution	Clearance	Half-Life
Abciximab	Chimeric Fab: mVar-hIgG1	CD41	8.4 L[58]	NA	0.29 days[137]
Adalimumab	hIgG1	TNFa	5.1–5.8 L[138]	9–12 mL/h[138]	14.7–19.3 days[138]
Alefacept	Lymphocyte function-associated antigen-3/ hIgG1(Fc)	CD2	6.5 L[c]	18 mL/h[c]	11.3 days[c]
Alemtuzumab	rCDR-hIgG1	CD52	NA	NA	12 days[139]
Basiliximab	Chimeric: mVar-hIgG1	CD25	7.5 L[140]	75 mL/h[140]	4.1 days[140]
Cetuximab	Chimeric: mVar-hIgG1	EGFR	3.5–5.2 L[c]	35–140 mL/h[a,c]	4.8 days[c]
Daclizumab	mCDR-hIgG1	CD25	5.9 L[c]	15 mL/h[c]	20 days[115]
Efalizumab	mCDR-hIgG1	CD11a	NA	70 mL/h[c]	NA
Etanercept	TNF-receptor/hIgG1(Fc)	TNFa	NA	120 mL/h[141]	4.0 days[141]
Gemtuzumab	mCDR-hIgG4	CD33	NA	265 mL/h[133]	1.9–2.5 days[133 a]
Ibritumomab tiuxetan	Murine IgG1	CD20	NA	NA	1.1 days[142]
Infliximab	Chimeric: mVar-hIgG1	TNFa	NA	NA	9.5 days[143]
Muromonab-CD3	Murine IgG2a	CD3	NA	NA	0.75 days[144]
Omalizumab	mCDR-hIgG1	IgE	5.5 L[c]	168 mL/h[c]	20 days[70]
Palivizumab	mCDR-hIgG1	RSV	NA	NA	19–27 days[145]
Rituximab	Chimeric: mVar-hIgG1	CD20	NA	NA	9.4 days[146 b]
Tositumab	Murine IgG2a	CD20	NA	68.2 mL/h[b,c]	2.7–2.8 days[b,c]
Trastuzumab	mCDR-hIgG1	HER2	3.6–5.2 L[147]	16–41 mL/h[147 a]	2.7–10 days[147 a]

Weight-normalized and body surface area-normalized values were converted based on 70 kg body weight and 1.73 m^2 body surface area. mVar-hIgG1, chimeric antibody comprised of murine variable regions and human IgG1 constant regions; hIgG1, human IgG1; rCDR-hIgG1, antibody comprised of rat complementary-determining regions grafted within a hIgG1 framework; mCDR-hIgG1, antibody comprised of mouse complementary-determining regions grafted within a hIgG1 framework; mCDR-hIgG4, antibody comprised of mouse complementary-determining regions grafted within a human IgG4 framework; TNFa, tumor necrosis factor alpha; EGFR, epidermal growth factor receptor; RSV, respiratory syncytial virus; HER2, human epidermal growth factor receptor 2; NA, information not found.

[a]Dose-dependent.

[b]Pharmacokinetics altered by treatment or extent of disease.

[c]Obtained from manufacturer prescribing information.

Table 2. FDA-Approved Polyclonal Immune Globulins and Antibody Fragments

Name	Species of Origin
CMV immune globulin	Human
Crotalidae immune Fab	Ovine
Digoxin immune Fab	Ovine
Hepatitis B immune globulin	Human
Intravenous gamma globulin	Human
Lymphocyte anti-thymocyte immune globulin	Equine, rabbit
Normal immune globulin	Human
Pertussis immune globulin	Human
Rabies immune globulin	Human
Rho(D) immune globulin	Human
Tetanus immune globulin	Human
Vaccinia immune globulin	Human
Varicella-zoster immune globulin	Human

and antigen binding sites (e.g., valence; ranging from 2 to 12). The most prevalent antibody isotype in man is IgG, which comprises approximately 85% of the immunoglobulin in serum. The majority of therapeutic antibodies are of the IgG isotype and, as such, this review will primarily focus on issues pertinent to this family of immunoglobulin.

Human IgG is comprised of four polypeptide chains, including two identical light chains and two identical heavy chains, where the chains are held together with a series of disulphide bonds (Fig. 2). Two light chain variants are found in man (e.g., κ or λ) each with a molecular weight of approximately 25 kDa. Additionally, the IgG heavy chains may be categorized into four subclasses: 1, 2, 3, and 4, each with a molecular weight of approximately 55 kDa. The intact IgG molecule has a valence of 2, and a total molecular weight

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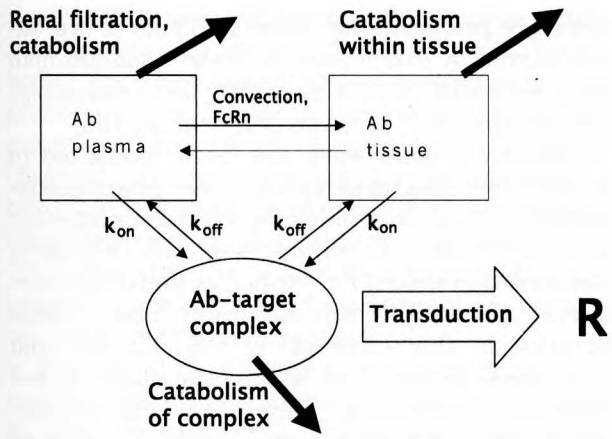


Figure 1. General scheme of antibody pharmacokinetics and pharmacodynamics. For many antibody drugs, primary determinants of disposition may be expected to include the rates of convective movement of antibody into tissue, convective return of antibody to blood *via* the lymphatic circulation, transport mediated by FcRn, interaction with target substances, and catabolism. Interaction with target substances may be often characterized with a second-order rate constant of association (k_{on}) and a first-order rate constant of complex dissociation (k_{off}). The relationship of the time-course of antibody–target complex formation to the time-course of antibody effects (R) may be determined by the kinetics associated with relevant transduction processes (e.g., Fcγ-receptor-mediated phagocytosis, antibody-dependent cell cytotoxicity, etc.).

of approximately 150 kDa. Each heavy chain and light chain contains a variable region and a constant region, and these regions may be considered to be responsible for allowing diversity in antigen binding and consistency in antibody structure, respectively. Fragments of IgG antibodies may be produced by enzymatic digestion (e.g., with papain or pepsin), or *via* recombinant methods. Although several different types of fragments have been described, the Fab fragment has found the most clinical application. Fab, which is an acronym meaning "fragment of antigen binding," has a valence of 1 and a molecular weight of ~50 kDa.

Antibodies, which are secreted from B-lymphocytes, may be characterized as "polyclonal" or "monoclonal," referring to the nature of the associated B-cell population. *In vivo*, exposure to antigen leads to the stimulation, diversification, and propagation of a large number of genetically distinct B-cells. Consequently, antibody in sera is polyclonal, with a distribution of affinity and specificity. However, it is possible to generate or isolate myeloma cells, which may replicate in culture and which may be cloned. Monoclonal antibodies, derived from a cloned population of cells, will have a unique structure (i.e., primary, secondary, and tertiary sequence) and unique affinity and specificity. Although polyclonal antibodies have been FDA-approved for clinical use (Table 2), the majority of antibodies on the market and the vast majority of antibodies in development are monoclonal preparations.

For a variety of methodological reasons, it is easier to generate cloned antibody producing cells of rodent versus human lineage. However, in general, administration of monoclonal rodent antibody to humans may be expected to lead to rapid generation of an immune response against the therapeutic antibody, which may lead to undesirable alteration of the pharmacokinetics and/or pharmacodynamics of the antibody. To overcome this issue, a number of approaches have been developed to "humanize" monoclonal rodent antibodies, including the development of chimeras that secrete monoclonal antibody with human constant regions and rodent variable regions, the generation of clones secreting "grafted" antibodies with human constant regions and with variable regions that are primarily comprised of human IgG sequence (i.e., with the exception of the complementary-determining regions (CDR) directly involved in antigen binding), and the development of transgenic mice that may be used to expedite

Table 3. Characteristics of the Human Immunoglobulin Isotypes*

	IgA	IgD	IgE	IgG	IgM
Molecular weight	160 kDa, 400 kDa	175 kDa	190 kDa	150 kDa	950 kDa, 1150 kDa
Molecular form	Monomer, dimer	Monomer	Monomer	Monomer	Pentamer, hexamer
Valence	2, 4	2	2	2	10, 12
Serum concentration (mg/mL)	1.5–2.6	0.04	0.0003	9.5–12.5	0.7–1.7
Serum half-life (days)[a]	6	3	2.5	23	5

*Data from Frazer and Capra.[2]
[a]Refers to the average half-lives of human immunoglobulins in normal human subjects.

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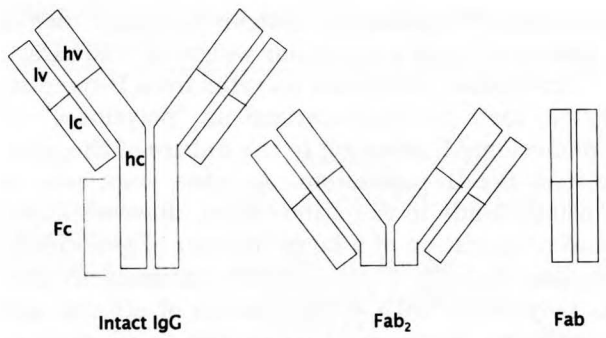


Figure 2. Structure of IgG and IgG fragments. Intact IgG is comprised of two heavy chains and two light chains. Heavy and light chains each contain a "constant" region (hc and lc) and a "variable" region (hv and lv). As noted in the text, the constant and variable regions allow consistency in antibody structure and diversity in antigen binding. The IgG molecule has a molecular weight of ~150 kDa and a valence of 2. The IgG fragments, Fab_2 and Fab, have molecular weights of ~110 and ~50 kDa, respectively.

the generation of monoclonal antibodies entirely comprised of human sequence.[3–5] As discussed below, the pharmacokinetics of antibody are highly dependent on structure, with clear differences found in comparisons of intact IgG and fragments, as well as with antibodies of different levels of humanization (e.g., fully-rodent, chimeric, CDR-grafted, and fully-human).

ANTIBODY PHARMACOKINETICS

Absorption

The majority of FDA-approved therapeutic antibodies are administered intravenously. The intravenous (iv) route has been preferred because this route of administration allows complete systemic availability, rapid delivery of antibodies to the systemic circulation, and achievement of high concentrations. Additionally, relative to other parenteral routes of administration, the iv route allows the administration of larger volumes. However, iv delivery has its limitations. This route is not convenient for patients, often requiring hospitalization, and thereby greatly increasing the cost of therapy. Rapid infusion of antibody may also precipitate adverse events and, consequently, there has been some interest in extravascular routes of antibody administration (e.g., oral, subcutaneous (sc), intramuscular (im), etc.). Of note, 6 of the 18 FDA-approved monoclonal antibody preparations listed in Table 1 are administered by extravascular routes (adalimumab (sc), alefacept (im), efalizumab (sc), etanercept (sc), omalizumab (sc), and palivizumab (im)).

Relatively little work has been conducted to assess the pharmacokinetics or pharmacodynamics of antibodies following oral administration in man.[6–8] The gastrointestinal tract provides a hostile environment for antibodies and other therapeutic proteins. Antibodies can undergo denaturation in the acidic pH of the stomach, and proteolytic degradation within the stomach and intestine. In addition, owing to their large molecular size and high polarity, antibodies may be expected to show minimal rates of diffusion through the gastrointestinal epithelium.

Nevertheless, intact immunoglobulins may reach systemic circulation by crossing the epithelial cells *via* paracellular transport or *via* receptor-mediated processes. For example, in the neonates of several species (mice, rats, dogs, cows, etc.), IgG is efficiently absorbed following oral administration.[9] This gastrointestinal absorption, which allows transmission of IgG from mother's milk to the newborn, is saturable, and is observed only in neonates (e.g., during the first 2–3 weeks postpartum for mice and rats). The receptor associated with this IgG transport, FcRn (i.e., the Fc-receptor of the neonate), has been isolated and cloned.[10,11] Interestingly, it is now known that FcRn is expressed in a wide variety of systemic tissues in adults, including endothelial cells of kidneys, liver, lungs, hepatocytes, intestinal macrophages, peripheral blood monocytes, and dendritic cells.[12–14] FcRn functions within systemic tissues to protect IgG from degradation,[9] and the receptor may be an important determinant of tissue disposition (discussed below).

In spite of the fact that FcRn has been detected within the human gastrointestinal epithelium, available data suggest minimal oral absorption of intact IgG in humans. For example, oral administration of purified IgG (100–800 mg/kg/day for 5 days) or an IgG–IgA preparation (600 mg/day for 28 days) did not lead to an increase in serum IgG concentrations in human infants.[7] As such, the utility of oral administration of antibody is likely limited to treatment of gastrointestinal infections.[6–8]

The systemic absorption of antibodies following sc or im administration most likely occurs *via* the lymphatic system. The highly porous lymphatic system allows the transport of macromolecules (>20000 g/mole) through the convective flow of the

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interstitial fluids. As the lymph fluid drains slowly into the vascular system, the absorption of antibodies from the site of administration may continue for hours. In animals and in man, maximal plasma concentrations of antibody are typically observed 1–8 days following sc or im administration,[15–17] and the time of attainment of maximal concentrations (t_{max}) has been indicated to be 131 ± 56 h, 69 ± 34 h, and 7–8 days within the prescribing information for adalimumab, etanercept, and omalizumab, respectively.

The extent of absorption may vary depending on the extent of presystemic antibody degradation by proteolytic enzymes. Proteolytic degradation may be saturable, leading to increases in bioavailability with increasing doses of antibody. In general, most investigations of antibody absorption following im or sc dosing have reported bioavailabilities ranging from 50 to 100%.[18–21] For example, following im administration, the bioavailability of alefacept (human IgG1 fusion protein) in healthy male volunteers was approximately 80%,[21] and humanized monoclonal anti-interleukin-5 IgG1 (SB-240563) demonstrated complete absorption ($F = 0.96–1.34$) following sc administration to monkeys.[15] Mean sc bioavailabilities for adalimumab and omalizumab are reported to be 64 and 62%, respectively. The most outstanding limitation of these routes may be that large doses of antibody may not be feasibly injected im or sc due to the relatively limited solubility of IgG (~100 mg/mL), and due to pain associated with administration of volumes greater than 2.5 or 5 mL (*via* sc and im injection, respectively). Of note, doses of 375 mg of omalizumab are routinely administered clinically, *via* three separate 1 mL sc injections. Thus, it may be concluded that im and sc dosing could be viable for therapeutic applications where rapid absorption is not necessary, and where antibody dose is not greater than ~500 mg.

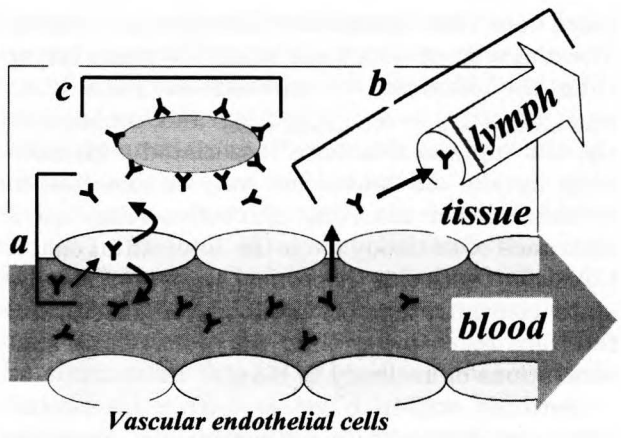


Figure 3. Antibody distribution. The cartoon represents several possible determinants of antibody distribution in tissue, including: (a) antibody entry into vascular endothelial cells *via* pinocytosis (e.g., fluid-phase endocytosis), and FcRn-mediated return to the blood or transport to the tissue interstitium; (b) entry of antibody into the tissue interstitium *via* convective transport through paracellular pores in the vascular endothelium, and elimination of antibody from tissue *via* convective transport with lymph fluid; and (c) binding of antibody to cellular antigens. As mentioned in the text, due to differences in the sieving effect of paracellular and lymphatic pores, it is reasonable to expect low antibody concentrations in interstitial fluid relative to blood. However, antibodies may be associated with large apparent volumes of distribution in cases where there is high affinity/high capacity binding of antibody in tissue.

Antibody Distribution

The rate and extent of antibody distribution will be dependent on the rates and extents of antibody extravasation within tissue, distribution within tissue, and elimination from tissue (Fig. 3). Extravasation may occur by paracellular or transcellular movement of antibody, which may proceed *via* convection (i.e., movement of antibody with fluid flow from blood to tissue), diffusion, or *via* receptor-mediated endocytosis. Due to the large molecular size and high polarity of immunoglobulin, it is unlikely that significant extravasation occurs *via* transcellular diffusion. As such, the majority of research on antibody distribution into tissues has focused on convective transport and on receptor-mediated endocytosis. Convective transport occurs as a result of fluid movement from blood to the interstitial space of tissues, which is primarily driven by the blood–tissue hydrostatic pressure gradient.[22–24] Additional determinants of convective transport of antibody include osmotic pressure gradients and the nature of paracellular pores (i.e., effective diameter, tortuosity, etc.). As pore size decreases and as the tortuosity of the paracellular path increases, it may be expected that there will be an increased resistance to the movement of macromolecular solute relative to solvent. Mathematical models of convective extravasation of antibody have characterized this "sieving effect" through the use of a reflection coefficient, σ, where mass transfer rates would equal the product of antibody concentration, solvent flow rates, and $(1-\sigma)$.[22,23] Fluid that enters tissue *via* extravasation is returned to

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blood *via* the lymphatic circulation system. The diameter of lymphatic vessels is much larger than the diameter of paracellular pores (i.e., $\sigma_{\text{paracellular pore}} \gg \sigma_{\text{lymphatic vessel}}$) and, consequently, the "uptake clearance" associated with antibody uptake *via* convection may be expected to be much lower than the convective elimination clearance of antibody from the interstitial space. Consistent with this theoretical argument, steady-state concentrations of antibody in interstitial fluid are much lower than the steady-state concentrations of antibody in blood.[25–27]

Antibody within blood or within interstitial fluid may enter cells *via* pinocytosis (e.g., receptor-mediated endocytosis, fluid-phase endocytosis). Receptor-mediated endocytosis may occur *via* Fcγ receptors (e.g., FcγRI, FcγRII, and FcγRIII), which are present on a variety of cells (e.g., monocytes, macrophages, B-lymphocytes, platelets, etc.), or *via* internalization of antibody bound to a membrane antigen (i.e., *via* the antigen-binding region of the immunoglobulin). Fluid phase endocytosis may be an important pathway for antibody internalization into cells, and it has been suggested that the majority of antibody extravasation occurs *via* fluid-phase endocytosis of antibody into vascular endothelial cells. Once within the acidic environment of cellular endosomes, IgG antibody may be bound by the Brambell receptor, FcRn.[9] FcRn-bound IgG is protected from intracellular degradation, and released to the plasma and/or interstitial space. *In vitro* investigations have shown that FcRn transports IgG in a bi-directional manner (i.e., in the apical–basolateral direction, and in the basolateral–apical direction);[28–30] consequently, there is some possibility that FcRn may be an important contributor to the transport of IgG from plasma to the interstitial fluid of tissues. However, the influence of FcRn on tissue distribution has not yet been investigated.

As with other drugs, the overall extent of distribution of therapeutic antibody is typically quantified by inferring the ratio of the mass of the antibody in the body at steady-state and the concentration of therapeutic antibody in plasma at steady-state (i.e., the apparent volume of drug distribution at steady state, Vss).[31] The mass of antibody in tissue will be determined by the mass of antibody in interstitial fluid and the mass of antibody bound to or internalized within cells. In cases where drug binds with high affinity to tissue sites, a significant fraction of antibody in the body may be bound in tissue, and large apparent volumes of distribution may be expected. Additionally, in cases where the binding capacity of tissue is limited, nonlinear distribution may be expected (i.e., where Vss decreases in a dose or concentration-dependent manner). Given that therapeutic antibodies are often developed for high-affinity binding to cellular antigens that are present in limited quantity, it might be reasonable to expect that reports of antibody pharmacokinetics would typically show large volumes, where Vss decreases as a function of increasing steady-state plasma concentrations.

Contrary to these expectations, in most reports of antibody pharmacokinetics, antibody Vss has been reported to be small (i.e., approximately equal to the plasma volume of the study subject) and concentration-independent (e.g., Table 1). However, it is worth noting that the vast majority of pharmacokinetic analyzes of antibody drugs (and of drugs in general) have estimated Vss *via* noncompartmental, moment approaches or *via* computer fitting with mammillary compartmental models (e.g., the 2-compartment open model, etc.). These analytical approaches are based on the assumption that the site of antibody elimination is in rapid equilibrium with plasma (i.e., it is assumed that all elimination is from the "central" compartment). This assumption may be valid for many antibodies, but incorrect for others (e.g., antibodies that bind to and internalize within cells in tissue sites). It is known that antibodies are catabolized in tissues throughout the body, including sites where the rate of antibody distribution is likely to be relatively slow. In the extreme case, where the rate of distribution of antibody from tissue to blood is much slower that the rate of antibody catabolism in tissue sites, Vss will be inappropriately inferred to be equal to the plasma volume (i.e., independent of the relationship of mass of antibody in the body to the concentration of antibody in plasma).

Antibody distribution has been investigated more directly by assessing the concentrations of antibody in tissues, where tissue samples are obtained by biopsy or necropsy. For most antibodies, tissue:blood concentration ratios are found to be in the range of 0.1–0.5.[23,32] Given that plasma volume is 3–5% of total body volume, antibody in plasma may be expected to comprise ~20–50% of antibody in the body. As such, the true Vss for most antibodies may be expected to be 2–5 times the plasma volume. However, in cases where antibody binds with high affinity to extravascular sites that are associated with high binding capacity, tissue:blood antibody concentration

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ratios may be much greater than 0.5. For example, Kairemo et al.[33] reported that the tissue concentrations of a monoclonal antibody against keratan sulfate were much greater than blood concentrations, with concentration ratios of 5.9, 4.3, 3.5, and 2.6 for lung, esophagus, kidney, and liver, respectively. Similarly, Danilov et al.[34] found very high tissue to blood concentration ratios for a series of antibodies directed against endothelial antigens. For example, PECAM-1, an anti-CD31 antibody, was associated with tissue:blood concentration ratios of 13.1, 10.9, and 6.4 for the lung, liver, and spleen, respectively. In these examples, and in other cases where there is high affinity, high capacity binding of antibody to tissue sites, there is a high likelihood that antibody distribution volume will be much greater than plasma volume (e.g., PECAM-1 likely has a true Vss that is $>15\times$ plasma volume). As such, it is important to note that reported values of antibody distribution volume might be erroneous (i.e., due to the application of methods with incorrect assumptions). Due to the possibility that antibody may be eliminated from sites that are not in rapid equilibrium with plasma, direct assessment of antibody concentration in tissue is advised (i.e., *via* biopsy or necropsy).

Antibody Clearance

Antibodies, like other substances, may be eliminated *via* excretion or *via* metabolism (e.g., catabolism). Owing to their large molecular size, little intact immunoglobulin is filtered by the kidney, and little antibody is excreted in the urine. Low molecular weight antibody fragments (e.g., Fab and Fv) are filtered; nevertheless, the majority of the filtered protein is not excreted, but reabsorbed and/or metabolized by proximal tubular cells of the nephron. Small quantities of immunoglobulin are found in bile, and billiary excretion accounts for approximately 3% of the elimination of IgA.[35] The vast majority of immunoglobulin is eliminated by catabolism.[36] Early investigations conducted with pooled antibody suggested that immunoglobulins are primarily metabolized within sites that are in rapid equilibrium with plasma; however, the exact anatomical locations of antibody catabolism have not been identified.[36]

As shown in Table 3, the serum half-life of IgG (23 days) is substantially longer than those associated with other immunoglobulin isotypes (2.5–6 days). The elimination of IgG is known to be concentration dependent, where half-life decreases as a function of increasing serum IgG concentrations.[36] In reviewing these characteristics of IgG pharmacokinetics, Brambell et al.[37] proposed in 1964 that IgG may be a substrate of a transport protein that mediates both neonatal absorption (discussed in "Absorption" above) and also protection from systemic elimination. Additionally, Brambell et al. hypothesized that the transport receptor might be saturated at high concentrations of IgG, thereby providing a mechanistic explanation for the observed increase in the elimination rate of IgG with increasing IgG concentrations. Subsequent research led to the isolation[10] and cloning[11] of the receptor that mediates IgG absorption in neonatal mice and rats, and this receptor was named "FcRn," the Fc-receptor of the neonate. Approximately 30 years after Brambell's hypothesis was published, several groups studied the systemic pharmacokinetics of IgG in FcRn knockout mice, and found that IgG elimination rate was increased 10–15 fold, with no change in the elimination of other immunoglobulins (e.g., IgA).[38–40] Subsequent research has demonstrated that the serum half-lives of IgG antibodies are directly related to their binding affinity for FcRn.[41,42] Thus, these studies confirmed Brambell's prediction that the same receptor that was responsible for IgG absorption in neonates (FcRn) was responsible for the protection of IgG from systemic elimination.

Figure 4 illustrates the potential role of FcRn in IgG catabolism. In this schematic, IgG is taken up

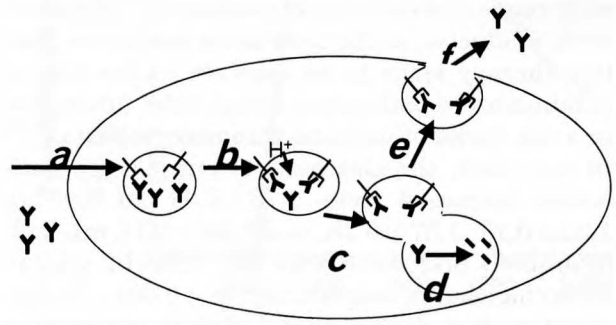


Figure 4. Proposed mechanism for IgG protection by FcRn. (a) IgG molecules enter cells (e.g., vascular endothelial cells, muscle cells, etc.) *via* pinocytosis (e.g., fluid-phase endocytosis); (b) H^+ enters the endosome, reducing pH, and IgG binds with FcRn; (c) the endosome fuses with the lysosome, but FcRn-bound IgG is not released to the lysosome; (d) unbound IgG is released to the lysosome and catabolized by proteases; (e) the endosome fuses with the plasma membrane, the FcRn–IgG complex is exposed to physiologic pH; and (f) IgG is released into plasma (or interstitial fluid).

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into endosomes of catabolic cells by fluid-phase pinocytosis. At physiologic pH, IgG has low affinity for FcRn. As the pH decreases in the endosome, the affinity of IgG for FcRn increases, and IgG can bind to the receptor, *via* the Fc portion of the IgG molecule. FcRn-bound IgG is returned to the central compartment, and unbound IgG proceeds to the lysosome and undergoes proteolytic degradation.

Owing to the large quantity of endogenous IgG that is present in the body, very large doses of therapeutic antibody would be required to produce significant alterations in serum IgG concentrations. For example, assuming an average steady-state distribution volume of 5–10 L for an average human subject and a "baseline" serum IgG concentration of 10 g/L, the steady-state mass of IgG in the subject may be estimated to be 50–100 g. As such, an iv IgG dose of 15 mg/kg would only increase the mass of antibody in the body by ~1–2%; additionally, immediately after administration, "peak" IgG plasma concentrations would be expected to be ~3% higher than the baseline concentration of IgG (i.e., assuming a plasma volume of 3 L). Based on the relationship of IgG concentration to the elimination rate of IgG, these small increases would lead to insignificant changes in the apparent elimination rate constant of IgG.[36]

However, very large doses of IgG may lead to substantial increases in the rate of elimination of exogenous and endogenous IgG. For example, iv administration of 1–2 g/kg of pooled human immunoglobulin has been used as a therapy for a wide range of autoimmune conditions.[43,44] Recent work conducted in this laboratory has shown that this therapy leads to an increase in the rate of elimination of pathogenic antiplatelet antibodies in a rat model of immune thrombocytopenia.[45,46] In our work, the clearance of antiplatelet antibodies increased from 0.78 ± 0.09 mL/kg/h to 1.28 ± 0.19, 1.37 ± 0.28, and 1.85 ± 0.19 mL/kg/h in animals pretreated with 0.4, 1, and 2 g/kg iv immunoglobulin, respectively ($p < 0.001$). Subsequent studies showed that 1 g/kg iv immunoglobulin administration increased the clearance of antiplatelet antibodies in control mice that expressed functional FcRn (i.e., from 5.2 ± 0.3 to 14.4 ± 1.4 mL/kg/day, $p < 0.001$); however, consistent with the hypothesis that this effect is mediated *via* saturation of FcRn, iv immunoglobulin treatment failed to increase the clearance of antiplatelet antibodies in knockout mice lacking FcRn expression.[47] Mathematical modeling has suggested that the observed increase in the elimination of antiplatelet antibodies may explain much of the observed effects of iv immunoglobulin therapy in the rat model of immune thrombocytopenia.[48] As such, it is conceivable that much of the efficacy of high dose immunoglobulin therapy in human autoimmunity is due to increased elimination of pathogenic autoantibodies.

Although little definitive work has been published regarding the sites of IgG elimination, it has been hypothesized that the processes involved in regulating IgG catabolism are diffuse processes involving many cells throughout the body.[36,38] Supportive of this hypothesis are the findings that FcRn α-chain mRNA is expressed in many tissues, and that FcRn has been detected in both murine and human endothelial cells. In light of these observations, Ghetie and Ward[49] suggest that endothelial cells may be the key cells involved in IgG catabolism. Also of interest were the findings of Haymann et al.,[50] that FcRn is expressed in human renal glomerular epithelial cells, leading to the suggestion that FcRn may play an important role in the re-absorption of filtered IgG, and hence the minimization of the role of urinary excretion as a route of IgG elimination.

As the Fc portion of the IgG molecule is the region of the molecule responsible for the long persistence of IgG, attempts have been made to increase the half-lives of various therapeutic proteins by coupling the protein to the Fc portion of an IgG molecule.[51] Additionally, this approach has been used with the goal of imparting to the therapeutic the accompanying effector function of the subclass of IgG that is used as the fusion partner.[52] Some success has been achieved with these fusion proteins, with etanercept and alafacept both reaching the market in recent years. Others have attempted to further increase the half-life of IgGs by engineering antibodies with increased affinity for the FcRn receptor,[41] though these attempts have not yet resulted in products on the market.

Early investigations of the pharmacokinetics of murine monoclonal antibodies in man revealed that murine IgG demonstrated a serum half-life of 1–2 days,[53] much shorter than that of human IgG (~23 day). It is now known that this pharmacokinetic observation is resultant from the low affinity of human FcRn for murine IgG.[54] Ober et al.[54] have shown that human FcRn shows high affinity for human, rabbit, and guinea pig IgG, but little or no affinity for mouse, rat, bovine, or sheep IgG. Typically, the half-lives of monoclonal IgG antibodies increase with the degree of "humanization,"

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with "fully-rodent" < rodent/human chimeric < CDR-grafted human < "fully human" (Table 1).

Therapeutic antibodies are often developed for high affinity binding to cellular antigens. As indicated above ("Antibody Distribution"), the interaction of antibody and cellular antigens may be an important determinant of antibody distribution. Additionally, depending on the nature of the target and the downstream events resultant from antibody-ligand binding, this interaction may also influence the clearance of the antibody. Several studies have suggested that this antigen–antibody interaction may be important to the overall rate of elimination of a particular antibody.[55–58]

In many cases, IgG elimination is likely dominated by affinity for the FcRn receptor, and the nature of and affinity for the specific target of the antibody. However, other factors may contribute to the rate of antibody elimination, including the immunogenicity of the antibody,[59] the degree and nature of the glycosylation of the antibody,[60–62] and the susceptibility of the antibody to proteolysis.[63] Further study is certainly warranted to better understand the relative importance of the various factors involved in antibody elimination.

Influence of Antimonoclonal Antibodies on Antibody Pharmacokinetics

The administration of therapeutic proteins, such as antibodies, may elicit an immune response that leads to the generation of endogenous antibodies that are directed against the therapeutic protein.[64] The potential for a therapeutic protein to lead to an immune response (i.e., the "immunogenicity" of the protein) appears to be dependent on a number of factors, including the dosing strategy (i.e., dose, route, and frequency of administration), the degree of aggregation of the therapeutic protein product, and the extent of similarity between the exogenous protein and the endogenous proteins in the recipient.[65] Immunogenicity is not well understood, and the immunogenicity of a therapeutic protein may not be reliably predicted. However, several studies have shown that immunogenicity increases with the quantity of aggregates in the dosing formulation.[66] Additionally, immunogenicity appears to be influenced by the dosing route, with increasing antibody response in the following rank order: iv < im < sc.[65] With regard to therapeutic IgG antibodies, it appears that immunogenicity decreases with the extent of IgG humanization (i.e., rodent IgG > chimeric IgG > CDR-grafted IgG > "fully-human" IgG). For example, Rebello et al.[5] found that 15 of 17 patients developed antibodies following treatment with a rat anti-CD52 antibody, but no detectable antibody responses were found following administration of a humanized derivative of the rat antibody to 12 patients.

Following the administration of an immunogenic protein, a measurable antiprotein antibody response is typically observed within 7–10 days.[67] In an interesting study, Gobburu et al.[68] assessed the kinetics of antibody induction following administration of tetanus toxoid to cynomolgus monkeys. Measurable antibody in plasma was found approximately 200 h following administration of the immunogenic protein, with peak antibody concentrations observed at approximately 400 h (Fig. 5). These findings are similar those of Richter et al., which showed that administration of lenercept, an IgG1 fusion protein, led to the development of antilenercept antibodies in rabbits and dogs (Fig. 6). Importantly, the Gobburu et al. study developed an indirect pharmacodynamic model, which was able to capture the time-course of the endogenous antibody response; similar models

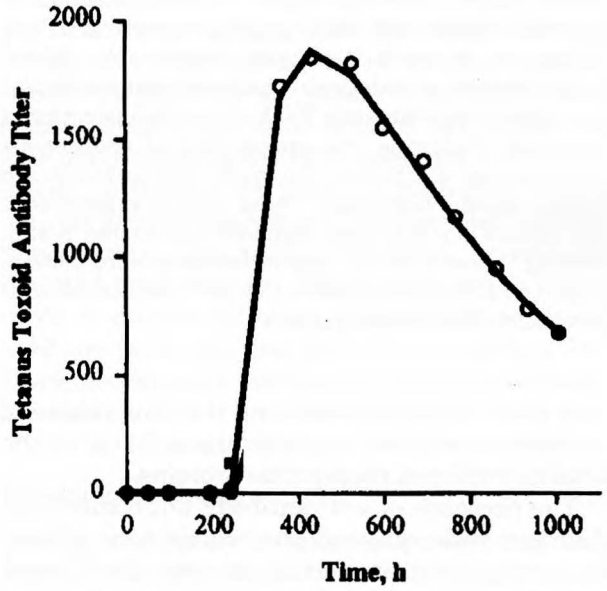


Figure 5. Time-course of endogenous antibody concentrations following administration of an immunogenic protein. Shown is the time-course of mean values of the plasma titer of three female cynomolgus monkeys following intramuscular (im) administration of tetanus toxoid. Time values are in hours. As shown, measurable antibody was found approximately 200 h following administration of the toxoid, with peak concentrations observed at ~400 h. Figure modified from Gobburu et al.[68] (reprinted with permission from the publisher).

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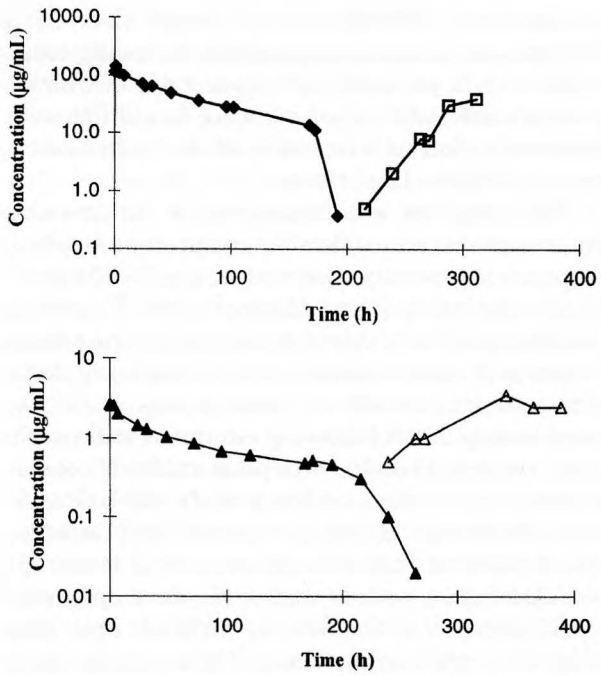


Figure 6. Pharmacokinetic effects of antilenercept antibodies. Shown is the time-course of lenecept (closed symbols) and endogenous antilenercept antibodies (open symbols) in a rabbit (upper panel) and in a dog (lower panel) following single, iv administration of lenercept at doses of 5 and 0.1 mg/kg, respectively. Time values are in hours. As shown, measurable plasma concentrations of endogenous antilenercept antibodies were found approximately 200 h after administration of lenercept. Following the appearance of endogenous antilenercept antibodies, lenercept concentrations in plasma rapidly decreased. These results suggest that endogenous antilenercept antibodies mediated a substantial increase in the rate of lenercept elimination. Figure modified from Richter et al.[74] (reprinted with permission from the publisher).

may be of value for predicting the time-course of endogenous antibody concentrations following the administration of therapeutic proteins.

The presence of anti-antibody antibodies may lead to a wide range of pharmacokinetic effects. In a recent review, Rehlander and Cho[69] have suggested that the pharmacokinetic effects of endogenous antibodies are dependent on the number of antigenic sites found on the therapeutic protein. In cases where endogenous antibodies are directed against only 1 or 2 sites on the therapeutic protein, the half-life of the therapeutic protein often approaches that of endogenous IgG (i.e., where interaction of induced IgG antibodies with the protein often increases the biologic half-life and concentrations of the therapeutic protein). Of note, this is similar to the effect seen for therapeutic monoclonal antibodies on the disposition of soluble targets (e.g., small molecule drugs, cytokines, proteins, etc.). For example, following the administration of omalizumab, a monoclonal IgG antibody directed against IgE, total IgE concentrations are found to increase 4–5 fold.[70] This finding is consistent with an increase in the half-life of IgE from its normal value of approximately 3 days to approximately 15 days (i.e., approaching the half-life of IgG).

On the other hand, in cases where endogenous antibodies are directed against three or more sites on the therapeutic protein, the therapeutic protein is often eliminated very rapidly (i.e., often producing dramatic reductions in the half-life of the therapeutic protein; e.g., Fig. 6). As such, the influence of endogenous antibodies on the disposition of therapeutic antibodies is complex and difficult to predict. However, it is reasonable to expect that with increased exposure to the therapeutic antibody (i.e., with increasing duration and frequency of dosing), there is an increasing probability for development of endogenous antibodies directed against multiple antigenic sites, and there is an increasing probability that endogenous antibodies will mediate rapid elimination of the therapeutic antibody.

Interspecies Scaling

Despite the large number of antibodies in development, only a handful of reports have been published in the primary literature addressing the use of nonclinical data to predict the clinical pharmacokinetics of antibodies. Both physiologically-based pharmacokinetic (PBPK) models and power models have been used to accomplish this interspecies scaling.[18,71–75]

Mordenti et al. first reported use of an allometric power model to scale therapeutic proteins, including a fusion protein comprised of portions of the CD4 molecule and the Fc portion of an IgG1 molecule. This analysis used a power model of the form $Y = a \cdot BW^b$, where Y is the PK parameter of interest, BW is the body weight, a is the allometric coefficient, and b is the allometric exponent.[71] Both the fit of the preclinical parameters (clearance and volume) and the prediction of clinical parameters were excellent for this IgG fusion molecule. Exponents for clearance and volume terms were very close to the "expected" values of 0.75 and 1, respectively. Subsequent to the study by Mordenti et al., others have reported the use of

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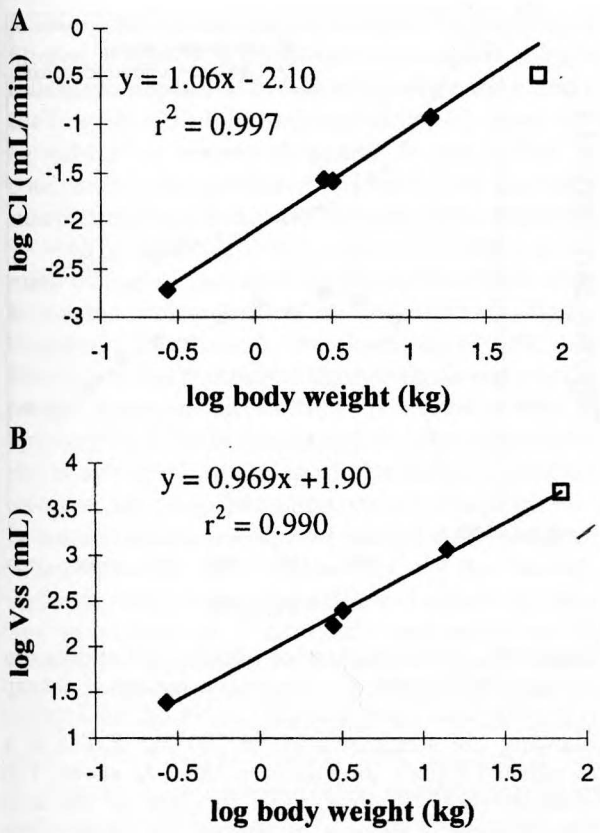


Figure 7. Allometric scaling of lenercept. Shown are the log values of clearance (upper panel) and volume of distribution at steady state (lower panel), presented in relation to log body weight. Closed symbols represent observed values in animals (rats, rabbits, cynomolgus monkeys, and dogs) with regression line for prediction; open symbols indicate the observed values in humans. Figure modified from Richter et al.[74] (reprinted with permission from the publisher).

power models to scale PK parameters for IgG,[18] Fab,[72] Fab_2,[75] and fusion proteins (Fig. 7),[74] and these reports have generally concluded that this type of approach was useful for IgG related molecules.

Another approach to interspecies scaling and the prediction of clinical pharmacokinetics of IgG, presented by Baxter et al.[73] in 1995, is the use of PBPK modeling. This approach involved building a physiologically-based model using data obtained in mice, and then using human physiologic parameters to predict human kinetics. As shown by Baxter et al., PBPK models may allow prediction of antibody levels in many tissues (including tumor). This is a significant advantage of the PBPK approach, particularly when one considers the difficulties associated with the inference of antibody distribution kinetics from antibody disposition in plasma (discussed above). Additionally, PBPK models can accommodate several complexities that power models are unable to deal with (e.g., effects of antibody affinity; nonlinear kinetics).

Although the few literature reports addressing interspecies scaling of antibodies would lead one to conclude that allometric power relationships may be adequate to predict clinical antibody pharmacokinetics, one must proceed with caution and consider all of the factors governing antibody kinetics before relying heavily on these predictions. In an attempt to scale IgG pharmacokinetics, one should consider: (1) possible species differences in antibody–antigen binding, (2) the impact of antigen binding on IgG kinetics, (3) possible differences in binding to the FcRn receptor between species, and (4) the immunogenic potential of the antibody in various species. Of note, species-specific parameters may be readily incorporated into PBPK models and, consequently, physiologically-based models may be ideally suited for predicting the influence of the factors listed above.

ANTIBODY PHARMACODYNAMICS

Antibodies may act by a wide variety of pathways to achieve a diverse array of pharmacological effects. To facilitate discussion of antibody pharmacodynamics, we have focused our review on four main applications of therapeutic antibodies, where antibodies are used to neutralize toxins (i.e., immunotoxicotherapy), mediate the destruction of cells, alter cellular function, or mediate targeted drug delivery. Each topic area is structured to discuss relevant caveats, cite approved antibody products, and provide examples of the pharmacodynamic analysis of selected antibody products.

Immunotoxicotherapy

Immunotoxicotherapy refers to passive immunization with antibody preparations to prevent or reverse toxicities associated with venoms, toxins, drugs, and endogenous ligands (e.g., cytokines, etc.). Several such antibody preparations have been FDA-approved, including monoclonal antibodies (e.g., adalimumab, infliximab, omalizumab), fusion proteins (e.g., entanercept), and purified, specific polyclonal antibody preparations (e.g., digoxin immune Fab, crotalidae polyvalent immune Fab). Such antibody preparations, hereafter collectively referred to as antiligand antibodies,

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have been used for a wide range of applications, including for treatment of snake, scorpion, and insect venom poisoning,[76] immunomodulation (i.e., as achieved with antibodies directed against cytokines), and for treatment of intentional or accidental overdose of small molecule drugs, such as morphine, digitalis, colchicine, and antidepressants.[77–80] Additionally, a range of preclinical and clinical investigations have examined the utility of immunotoxicotherapy for diverse applications such as for the reversal of septic shock induced by bacterial endotoxin,[81] anticoagulation,[82,83] and for the prevention of systemic toxicities associated with anticancer agents (e.g., doxorubicin, methotrexate, etc.).[84–86]

Passive immunization with antiligand antibody achieves effects *via* altering the pharmacokinetics of the target ligand. The changes in the disposition of the ligand are governed by the disposition of the antibody, affinity of the antibody, the molar dose ratio of the antibody:ligand, and the dosing protocol employed. In most cases, the distribution volume and the rate of clearance of antiligand antibodies will be much lower than the distribution volume and clearance of the target ligand and, consequently, antiligand antibodies often induce dramatic alterations in ligand disposition. Typically, iv administration of antiligand antibodies to an intoxicated subject may be expected to lead to rapid binding of ligand in plasma, decreasing unbound ligand concentrations in plasma. This binding will shift the tissue-to-plasma ratio of unbound ligand concentration, leading to a redistribution of ligand from tissue to plasma.[87,88] Due to the dual effects of antibody binding and ligand redistribution, administration of antiligand antibodies will often lead to a large increase in ligand concentration in plasma, reductions in the unbound concentration and free fraction of ligand in plasma, and large decreases in ligand concentrations in tissue,[89] often allowing a rapid attenuation of the pharmacological effects of the ligand. For example, within minutes of administration, antidigoxin immune Fab increases plasma digoxin concentrations by 10–100 fold and decreases unbound plasma digoxin concentrations below detectable limits, allowing rapid reversal of life-threatening digoxin intoxication (Fig. 8).[90]

Relatively little research has been conducted to assess the fate of antibody–ligand complexes. However, the interaction of small-molecule ligands with antiligand antibody does not appear to alter the disposition of the antibody. As such, the fate of antibody–ligand complexes may be predicted from what is known of the disposition of antibodies.[91] That is, it is likely that IgG–ligand complexes are processed by cells throughout the body (i.e., in the vascular endothelium, muscle, liver, etc.). Following endocytosis, antibody–ligand complexes will be exposed to acidic pH within the endosome, which may lead to ligand dissociation. Depending on the characteristics of the compound, ligand may be digested within the lysosome (e.g., as suggested by Junghans and Anderson[38] to explain the differential catabolism of tac protein and anti-tac antibodies in mice). However, it is plausible that lipophilic small-molecule ligands may diffuse into the cellular cytoplasm and then diffuse out of the cell (e.g., gaining re-entry into the interstitial fluid or plasma). In the case of antibody fragments (e.g., Fv or Fab), a substantial fraction of the antibody–ligand complex may be filtered by the glomerulus. For example, greater than 65% of digoxin immune Fab appears to be eliminated by the kidney in patients with normal renal function,[90] and renal impairment leads to increases in Fab elimination half-life and decreases in clearance.[92,93] However,

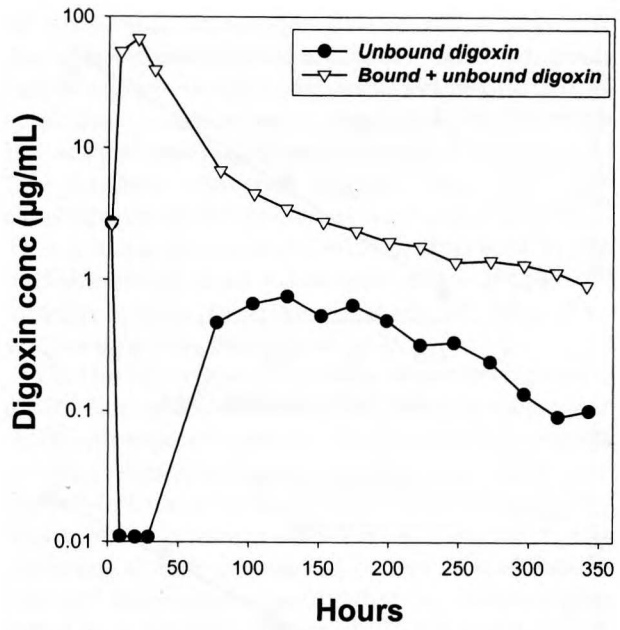


Figure 8. Pharmacokinetic effects of antidigoxin immune Fab. Shown are the concentrations of digoxin in plasma (total, open triangles; unbound, solid circles) following the administration of 120 mg of Fab to a 75-year-old digoxin-intoxicated patient. As shown, Fab administration led to a rapid increase in the total concentration of digoxin in plasma (i.e., from ~3 to ~90 µg/L) and a rapid decrease in the concentration of unbound digoxin in plasma (i.e., from ~3 µg/L to levels below the limit of assay detection). Figure digitalized from Ujhelyi and Robert.[90]

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following filtration, it appears that the majority of filtered antibody is digested by proximal tubular cells of the nephron.[94,95] This digestion may lead to the release of Fab-bound ligand, which then may be voided as unbound ligand in the urine or, potentially, reabsorbed into blood. The excretion rate of drug bound to antibody may be described as: $\frac{dXe}{dt} = CL_A \cdot ABL \cdot (1 - Rf)$, where *Xe* refers to the mass of ligand excreted in the urine, *ABL* refers to plasma concentration of the antibody–ligand complex, CL_A refers to the clearance of ABL, and *Rf* refers to the fraction of ligand that is reabsorbed to the systemic circulation. In cases where *Rf* approaches 1, little ligand will be eliminated from the body *via* elimination of the ligand–antibody complex. On the other hand, where *Rf* approaches 0, a substantial fraction of ligand may be eliminated through the elimination of the ligand–antibody complex, and antiligand antibody therapy may lead to a dramatic reduction in the cumulative exposure to unbound ligand (which may be quantified as the area under the unbound ligand plasma concentration vs. time curve, fAUC).[86,96] Several studies with antiligand Fab fragments have demonstrated increased urinary elimination of ligand,[88,96,97] and Balthasar and Fung[96] have shown that administration of antimethotrexate Fab leads to a decrease in methotrexate fAUC in rats.

The pharmacokinetic effects induced by antiligand antibodies often lead to desired antagonistic activity. However, the administration of antiligand antibodies occasionally leads to unexpected increases in the magnitude of effects produced by the target ligand. For example, Byrnes-Blake et al.[98] have reported that administration of a monoclonal antimethamphetamine antibody reduced methamphetamine-induced locomotor effects following low-dose methamphetamine (0.3 and 1 mg/kg), but increased methamphetamine-induced locomotor effects following a higher dose (3 mg/kg). Many investigations have shown that anticytokine antibodies can produce similar "agonist-like" effects.[99–101] May et al.[99] demonstrated that administration of anti-interluekin antibodies led to a 30% increase in interleukin-6 induced fibrinogen levels in mice, and anti-interleukin-3 antibodies increased mucosal mast cell number by 16-fold compared to results found in mice treated with interleukin-3 alone.[100] Additionally, monclonal antiamanitin IgG and Fab increased mortality resulting from α-amanitin exposure in mice,[102] and, in a clinical study assessing the utility of an anti-TNF fusion protein for treatment of septic shock, the anti-TNF therapy led to a dose-dependent increase in mortality, from 30% in placebo-treated patients to 53% in patients treated with 1.5 mg/kg of the fusion protein.[103]

The observed "agonistic" effects of antiligand antibodies may be due to antibody-induced alterations in the tissue exposure and/or due to antibody-induced alterations in the time-course of ligand exposure. For example, antiamanitin Fab therapy has been shown to greatly increase renal concentrations of α-amanitin, presumably resultant from the filtration and metabolism of Fab–amanitin complexes by the kidney.[102] Based on histological assessments, the authors concluded that increases in amanitin-induced toxicity were precipitated by increased delivery of amanitin to the kidney following antiamanitin antibody therapy. Additionally, antibody–ligand complexes typically demonstrate much longer elimination half-lives relative to that of the ligand (i.e., in the absence of antibody). As such, administration of antiligand antibodies will often lead to prolonged exposure to low concentrations of ligand. Depending on the pharmacodynamic characteristics of the ligand, this change in the time-course of ligand exposure may lead to increased toxicity. Work conducted in this laboratory with monoclonal antimethotrexate Fab and antimethotrexate IgG has shown that these antibody preparations could both increase and decrease methotrexate-induced toxicity in mice, with results dependent on the dosing regimens employed for the drug and for the antidrug antibodies.[86] Results were consistent with predictions made based on the effect of antibody on the time-course of methotrexate exposure.

Given the complexities associated with the effects of antiligand antibodies on ligand disposition and on ligand effects, there has been substantial interest in the use of PK/PD modeling to guide the development of immunotoxicotherapies. For example, Balthasar and Fung[85] utilized a pharmacokinetic model to predict the effects of antidigoxin Fab on the disposition of digoxin in mice. Consistent with antibody–antigen binding theory, the model incorporated second-order Fab–digoxin association and first-order dissociation of the antibody–drug complex, and the model allowed prediction of the influence of antibody affinity on antibody-induced alterations in digoxin tissue exposure. Similar models were later employed to predict the effects of polyclonal and monoclonal antimethotrexate IgG and Fab on methotrexate disposition in rats and mice.[86,96] In the later work, the effect of antibody on

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methotrexate disposition was linked to a pharmacodynamic model for methotrexate-induced toxicity, and the resultant PK/PD model accurately predicted the observed agonistic and antagonistic effects of monoclonal antimethotrexate IgG and Fab.[86,104] Racine-Poon et al.[105] utilized a standard direct-effect, inhibitory E_{max} model to characterize the effects of a monoclonal anti-IgE antibody on IgE disposition. This model was helpful for the prediction of effects following multiple-dose therapy (i.e., based on fittings obtained following single dose administration of the anti-IgE antibody). Similar to the modeling work of Balthasar and Fung, bimolecular antibody–ligand association models have been used to characterize the effects of a humanized monoclonal anti-Factor IX antibody on Factor IX activity and on the time-course of activated partial thromboplastin time in rats, monkeys, and man.[82,83,106]

Destruction of Target Cells

Several therapeutic antibodies have been developed to mediate the destruction of target cells (e.g., lymphocytes, cancer cells, bacteria, etc.). In general, two main strategies have been pursued, where antibodies are either used to opsonize target cells for subsequent destruction by "effector" mechanisms of the immune system, or where antibodies are used as a targeting vector to enhance the delivery of cytotoxic agents (e.g., chemotherapeutics, toxins, radioisotopes, etc.). "Antibody-Directed Drug Delivery," will discuss the latter strategy, whereas the present section will focus on the use of antibodies for opsonization of target cells. Successful antibody products include the FDA-approved muromonab-CD3, alemtuzumab, and rituximab. Muromonab-CD3 is a monoclonal, murine IgG2a antibody that is directed against CD3, an antigen present on the plasma membrane of T-lymphocytes.[107] Administration of the antibody leads to a rapid depletion of T-lymphocytes in peripheral blood, which provides desirable immunosuppression for treatment of acute allograft rejection. Alemtuzumab, a humanized murine monoclonal IgG1 antibody directed against a lymphocyte membrane glycoprotein (CD52), has been approved for treatment of B-cell chronic lymphocytic leukemia. Similarly, rituximab has been developed to bind to CD20, a B-lymphocyte membrane protein. Rituximab-opsonized B-cells are rapidly destroyed and, consequently, the antibody serves as an effective therapy for B-cell nonHodgkin's lymphoma.

Several effector mechanisms are likely responsible for cellular destruction following antibody opsonization, including antibody-dependent cell cytotoxicity, complement-mediated cellular lysis, complement-mediated phagocytosis, Fcγ-receptor-mediated phagocytosis, antibody-dependent cellular lysis, and apoptosis. The ability of antibodies to mediate cellular elimination *via* these mechanisms in man is dependent on a number of factors, including antibody density on the cellular surface, antibody isotype, antibody subclass, the species of antibody origin, and the extent of humanization. For example, complement-mediated phagocytosis and complement-mediated lysis are facilitated by the fixation of the complement factor C1q. Activation of C1q, which contains six Fc-binding sites/molecule, requires the occupation of at least two of the six sites. IgM immune complexes contain 5–6 activated Fc regions; consequently, a single IgM immune complex can activate C1q and initiate the complement cascade.[108] However, IgG antibodies contain only one Fc-region, and complement activation requires a high particle surface-density of IgG (i.e., ~1 IgG/800 nm^2).[108] Cells that express Fcγ receptors (e.g., natural killer cells, monocytes, macrophages) can initiate antibody-mediated destruction of cells opsonized with IgG antibodies; however, the affinity of Fcγ-receptors for IgG is subclass dependent.[109] For example, IgG2 shows much less binding to macrophage Fcγ receptors relative to IgG1, IgG3, or IgG4. As such, the pathways and the kinetics of antibody-mediated cellular destruction are numerous and complex; nonetheless, there is promise that antibody pharmacodynamics may be predicted and engineered through the growing knowledge of relationships between antibody structure and effector mechanisms.[110,111]

To date, relatively little work has been performed to model the pharmacodynamics of antibody-mediated elimination of cells. For example, to our knowledge, PK/PD models have not been presented for muromonab-CD3, altemuzumab, or rituximab. However, Sharma et al.[112] have reported the pharmacodynamic modeling of keliximab and clenoliximab, which are monoclonal, monkey/human chimeric antibodies directed against the CD4 antigen on T-lymphocytes. Keliximab and clenoliximab share the same parenteral antibody and, thus, demonstrate identical variable regions; however, keliximab is an IgG1 antibody and clenoliximab is an IgG4. As such, keliximab may be expected to be more efficient in eliminating T-cells *via* antibody-dependent cell

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lysis and *via* complement activation. In their approach, Sharma et al. employed an indirect-effect pharmacodynamic model[113] to relate clenoliximab and keliximab concentrations to alterations in the percentage of circulating CD4+ T-lymphocytes in transgenic mice.[112] Each antibody was assumed to reduce the percentage of CD4+ T-cells by stimulating cellular destruction, which was characterized with an E_{max} function (i.e, $\frac{dR}{dt} = k_{in} - k_{out} \times \left(1 + \frac{S_{max} \times C}{SC_{50} + C}\right) \times R$, where R refers to the percentage of CD4+ T-cells, k_{in} and k_{out} refer to the rate constants associated with the production and loss of CD4+ T-cells, and where S_{max} and SC_{50} are pharmacodynamic parameters associated with antibody effects on CD4+ T-cell elimination).[112] This model allowed good fitting of their data, and facilitated a quantitative comparison of the efficiency of IgG1 and IgG4 antibody-mediated destruction of CD4+ T-lymphocytes (Fig. 9). A similar model has been employed by Hansen and Balthasar[48] to characterize antibody-mediated thrombocytopenia in rats.

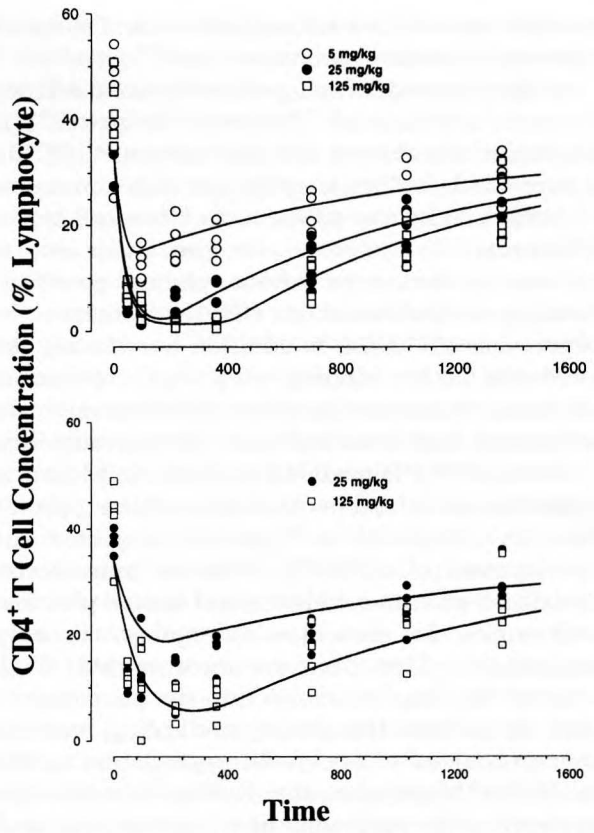


Figure 9. Pharmacodynamic effects of keliximab and clenoliximab in transgenic mice. Shown are the time-courses of the percentage of CD4+ lymphocytes following single-dose, iv administration of keliximab and clenoliximab, which are antibodies directed against the CD4 antigen (keliximab data, upper panel; clenoliximab data, lower panel). Time values are in hours. As shown, administration of anti-CD4 antibodies led to a rapid reduction in the percentage of CD4+ lymphocytes. The data were well-characterized by an indirect-effect pharmacokinetic/pharmacodynamic model, as described in the text. Figure modified from Sharma et al.[112] (reprinted with permission from the publisher).

Alteration of Cell Function

Several FDA-approved antibodies, including basiliximab, daclizumab, abciximab, efanizumab, and muromumab-CD3, may achieve effects by altering cell function. Additionally, several non-marketed antibodies have shown effects on cell function in nonclinical or early clinical studies, including clenoliximab and 5c8.

Abciximab is a chimeric monoclonal antibody Fab fragment targeted to the GPIIb/IIIa receptor on platelets. Abciximab is indicated as an adjunct to percutaneous coronary intervention for the prevention of cardiac ischemic complications. Binding of abciximab to platelet GPIIb/IIIa receptors inhibits the binding of adhesive molecules such as fibrinogen and von Willebrand factor, and prevents platelet aggregation. Nonclinical and clinical studies have shown that blockade of $\geq$80% of GPIIb/IIIa receptors is necessary for maximal inhibitory effects. Clinical pharmacodynamic endpoints have generally included *ex vivo* platelet aggregation studies and prolongation of bleeding time. Lacking the Fc portion of the IgG, abciximab binding to platelets does not lead to antibody-dependent thrombocytopenia in most patients. In contrast, the full IgG molecule from which abciximab is derived (7E3) can cause significant thrombocytopenia in animals.[45]

Another cell receptor target for FDA approved antibody products is the IL-2 receptor alpha chain (IL-2Rα), which is targeted by both daclizumab and basiliximab.[114,115] Daclizumab is a humanized IgG1,[115] whereas basiliximab is a chimeric human/mouse IgG1.[114] Although antiIL-2Rα therapy with either of these agents leads to a decrease in circulating T-lymphocyte levels, the mechanism of action of these agents is thought to be inhibition of IL2-mediated activation of lymphocytes. Anti-IL-2R therapies are indicated for the prevention of allograft rejection. As for abciximab, pharmacodynamic evaluations of anti-IL-2R antibodies tend to focus on saturation of cellular receptors. Literature reports have characterized the duration of receptor saturation following antibody administration, but attempts to correlate duration of

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receptor saturation with response (lack of allograft rejection) have not been successful.[116]

A third example of an agent that acts *via* cellular receptors is efalizumab. Efalizumab targets CD11a, a subunit of leukocyte function antigen-1. CD11a is expressed on all leukocytes, and efalizumab acts to inhibit leukocyte adhesion to other cell types. Efalizumab is indicated for the treatment of chronic moderate to severe plaque psoriasis. Binding of efalizumab to CD11a decreases the expression of CD11a, in addition to reducing the available CD11a binding sites. Again, principal pharmacodynamic endpoints with efalizumab are extent and duration of decreased CD11a availability.

Several PK/PD models have been developed to characterize antibodies that act to alter cellular function. Mager et al.[58] recently reported the development of a PK/PD model to characterize abciximab pharmacokinetics and *ex vivo* pharmacodynamics in patients undergoing coronary angioplasty. This analysis incorporated drug-receptor binding principles into the pharmacokinetic model and utilized a sigmoidal E_{max} model to characterize *ex vivo* platelet aggregation in the presence of 20 μM adenosine diphosphate. As might be expected for antibodies or fragments that bind to cellular antigens, a model that incorporated receptor-mediated disposition was able to describe the data well.

PK/PD modeling was also used to characterize human anti-CD11a antibody plasma concentrations and CD11a expression on CD3-positive lymphocytes in chimpanzees and in subjects with psoriasis.[57] Two models were developed in chimpanzees, and both models provided good fits of the data. As a noncompartmental analysis revealed that CL was not constant across dose levels, the first model incorporated a Michaelis–Menten clearance term into the pharmacokinetic equations. The second model added a complexity to account for CD11a concentrations in the Michaelis–Menten clearance term for the antibody, to be more consistent with the proposed hypothesis of receptor-mediated clearance. Both models utilized an indirect-response relationship to describe CD11a turnover. Dose-ranging studies were then conducted in humans, and a population PK/PD approach was used to characterize the data using both models. Based on fitting to the available data, it was not possible to determine which model was most appropriate; however, the second was more fully mechanistic in nature.

These two examples of PK/PD modeling of antibody effects on cellular processes demonstrate some of the complexities involved in attempting to characterize the concentration–response relationships for antibodies or similar compounds that bind with high affinity and specificity to capacity-limited cellular targets. Receptor-mediated disposition may be apparent for many of these types of agents, especially at low concentrations. Models that account for antibody–target interactions may be most consistent with current understanding regarding how these agents produce their desired effects, and may be most useful for simulation and exploration of the importance of various aspects of the models.

Antibody-Directed Drug Delivery

From the advent of hybridoma technology in 1975,[117] there has been hope that the specificity of monoclonal antibodies may be used within drug delivery strategies to improve the selectivity of drug therapy. Several approaches have been investigated in preclinical and clinical studies, involving the use of: (a) antibody–drug or antibody–radioisotope conjugates, where the drug or radioisotope is chemically linked to antibody,[118] (b) antibody–carrier conjugates, where a drug-carrier (e.g., liposome) is linked to antibody,[119,120] and (c) antibody–enzyme conjugates, where the enzyme is used to convert an inactive prodrug to an active drug.[121,122]

In addition to the general complexities associated with the use of antibody drugs (e.g., immunogenicity, high cost, etc.), the use of antibodies for drug delivery has been subject to a range of additional difficulties that likely contribute to the relative lack of success in this area. First, the vast majority of work done on antibody-directed drug delivery has attempted to increase the selectivity of cancer chemotherapy for the treatment of solid tumors. Due to the nature of uncontrolled cellular growth, solid tumors often are devoid of functioning lymphatic vessels.[123] Lymph drainage is thus impaired, which leads to the development of increased intrastitial hydrostatic pressure (i.e., relative to that found in healthy tissue).[123] The increased intrastitial pressure in tumors minimizes the blood-to-tissue hydrostatic pressure gradient, which effectively reduces the rate of fluid movement from blood to tumor tissue and thereby reduces the rate of antibody delivery to tumor *via* convection.[124] As such, despite desirable binding of antibody to tumor antigens, antibody distribution to solid tumors may be much less than anticipated due impaired convective delivery of antibody to the

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tumor. Additionally, tumor sectioning and autoradiography have shown that, following extravasation, antibody therapies often fail to distribute to all areas of solid tumors (i.e., as needed for eradication of all tumor cells).[124] This heterogeneous intra-tumoral distribution profile is likely due to low rates of antibody diffusion within the tumor interstitial fluid, convective flow of antibody from tumor to surrounding healthy tissue, and due to a possible "binding-site" barrier.[125,126] That is, high affinity binding of antibody to tumor cells in close proximity to blood vessels may impede further distribution of antibody within the tumor.

In spite of the complexities listed above, the application of antibodies for drug delivery remains an active area of research. Recent work focusing antibody-directed drug delivery to hematologic cancers (e.g., lymphomas, leukemias, etc.) has yielded impressive results. For example, ibritumomab tiuxetan, which employs an anti-CD20 monoclonal antibody to enhance the delivery of a radioisotope (^{90}Y), has been recently FDA-approved for treatment of B-cell nonHodgkin's lymphoma.[127,128] Additionally, gemtuzumab ozogamicin, an anti-CD33 antibody conjugated to the chemotherapeutic calicheamicia, has been FDA-approved for treatment of acute myeloid leukemia.[129,130] In each case, an antibody is used to increase the tumor-selectivity of a cytotoxic therapy (e.g., ^{90}Y or calicheamicia). The pharmacodynamics of such therapies may be expected to be determined by the intrinsic cytotoxic activity of the drug or radioisotope, antibody affinity for the target antigen, antigen density on the target cells, antigen density in plasma (i.e., "shed" antigen), and antigen density on nontarget cells. In the case of antibody-directed delivery of drugs (e.g., calicheamicia), pharmacodynamics will be also influenced by the rate and extent of antibody–antigen internalization and by the rate and extent of intracellular drug release from the antibody. In the case of radioisotopes (e.g., ^{90}Y), internalization of antibody and release of the isotope will not be necessary to achieve cytotoxic effects, due to the path length of the emitted particle.[131] However, internalization and radioisotope release remain to be important determinants of the pharmacodynamics of antibody–radioisotope immunoconjugates, as these processes, in combination, may lead to the loss of a substantial fraction of the radioisotope from the tumor site. This phenomenon may be of particular concern for conjugates employing ^{131}I, as this radioisotope may be rapidly released following dehalogenation reactions. In addition to the loss of radioactivity at the desired site, release of the isotope may lead to increased risk for undesirable toxic effects (e.g., hypothyroidism following ^{131}I-antibody therapy).[132]

There are relatively few reports of the use of mathematical modeling to characterize the pharmacokinetics and pharmacodynamics of antibody-directed drug therapy in patients. For example, PK/PD models have not been presented for application to predict or characterize effect versus time data for either ^{90}Y-ibritumomab or gemtuzumab ozogamicin. Clinical investigation of the pharmacokinetics and efficacy of gemtuzumab ozogamicin have shown that the clearance and apparent volume of antibody distribution are decreased following a second dose of antibody (i.e., relative to values found following the initial dose of gemtuzumab ozogamicin).[133] Given these pharmacokinetic results and the knowledge that the antibody leads to the destruction of cells expressing the CD33 antigen, it is reasonable to speculate that gemtuzumab ozogamicin is subject to target-mediated disposition, where the distribution and elimination of the antibody are influenced by the interaction of antibody with target cells.[55,134] This phenomenon would lead to a complex relationship between gemtuzumab ozogamicin exposure and effect, as the pharmacological effect of the antibody would lead to alterations in antibody disposition. As such, it is not entirely surprising that Dowell et al.[133] reported that no relationship was observed between antibody exposure (i.e., area under the plasma concentration vs. time curve) and patient response. Detailed modeling may be required for appropriate characterization of gemtuzumab ozogamicin PK/PD.

Although relatively little modeling of clinical data has been published, several groups have utilized PK/PD modeling to characterize data obtained from preclinical investigation of antibody-directed drug delivery and/or to predict antibody-directed drug delivery in man. In an interesting example, Zhu et al. utilized a physiologically-based simulation approach to investigate relationships between antibody dose and antibody uptake into tissue, to compare radio-immunoconjugates that employ intact IgG and IgG fragments for utility in therapy and for tumor imaging, and to assess optimal combinations of antibodies and radionuclides.[135] The modeling approach allowed consideration of a variety of physicochemical and physiological variables, including antibody molecular weight, affinity for antigen, and antigen density in tumor tissue and in healthy tissues.

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The authors concluded that a Fab fragment would be most useful for radio-immunodetection, and that optimal radio-immunotherapy would be provided by a Fab_2 fragment coupled with a long half-life beta-emitter. The basic structure of their physiologically-based model is well-suited for adaptation to the prediction and characterization of the PK/PD of additional immunoconjugates.

CONCLUSIONS/SUMMARY

Advances in the field of biotechnology have led to the development of techniques that have enhanced the rate of antibody discovery, reduced the risk of antibody immuno-toxicity, and increased the feasibility of large-scale antibody production. As a result of these advances, there has been a dramatic increase in the development of antibodies as drugs, and some investigators have reported that there are more than 700 antibody preparations in clinical trial.[136] However, several obstacles have not yet been overcome, and these obstacles continue to limit the successful use of antibodies to treat human disease. Continued research into the mechanisms and sites of antibody catabolism is needed, and such work may lead to new strategies to improve antibody bioavailability following oral, im, or sc dosing. Knowledge gleaned from research in pharmacogenomics and pharmacogenetics may expedite the development of individualized antibody therapies with enhanced selectivity and reduced toxicity. Improvements in our understanding of the interaction of therapeutic antibodies with the immune system may facilitate the engineering of antibody pharmacodynamics, and may also lead to new approaches to reduce antibody immunogenicity. Due to the interesting and complex nature of antibody disposition, and due to the wide range of potential mechanisms of antibody effects, the investigation of antibody PK/PD is a vibrant and exciting area of research. There is substantial promise that improvement in the present understanding of antibody PK/PD will lead to dramatic advances in the development of antibody drugs.

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