Review Article

Biodistribution Mechanisms of Therapeutic Monoclonal Antibodies in Health and Disease

Mohammad Tabrizi,[1,3] Gadi Gazit Bornstein,[2] and Hamza Suria[1]

Abstract. The monoclonal antibody market continues to witness an impressive rate of growth and has become the leading source of expansion in the biologic segment within the pharmaceutical industry. Currently marketed monoclonal antibodies target a diverse array of antigens. These antigens are distributed in a variety of tissues such as tumors, lungs, synovial fluid, psoriatic plaques, and lymph nodes. As the concentration of drug at the proximity of the biological receptor determines the magnitude of the observed pharmacological responses, a significant consideration in effective therapeutic application of monoclonal antibodies is a thorough understanding of the processes that regulate antibody biodistribution. Monoclonal antibody distribution is affected by factors such as molecular weight, blood flow, tissue and tumor heterogeneity, structure and porosity, target antigen density, turnover rate, and the target antigen expression profile.

KEY WORDS: biodistribution; monoclonal antibodies; pharmacokinetics and pharmacodynamics; tumor penetration.

INTRODUCTION

Similar to their small molecule counterparts, biodistribution of antibody-based therapeutics is a key consideration that can be modulated to impact the ensuing *in vivo* pharmacological effect(s). Understanding factors associated with antibody biodistribution allow for the intelligent design of therapeutic candidates that colocalize within the relevant effect compartment. Manipulation of candidate biodistribution is particularly useful in the development of next-generation antibody-based therapies. Given increasing competition among validated therapeutic targets such as tumor necrosis factor (TNF), epidermal growth factor receptor, vascular endothelial growth factor, and CD20, novel attributes may be engineered for the design of the next-generation candidates to permit commercial differentiation against the marketed predecessors. Differences in biodistribution between antibody-based therapeutics to the same target can result in a competitive advantage for one product *versus* another, and therefore, next-generation leads often are centered on leveraging such improvements to gain market penetration. For example, the current cadre of anti-TNF agents illustrates various biodistribution-related properties that may differentiate the next-generation antibody-based therapeutics. While full-length immunoglobulin G

(IgG) anti-TNF agents are primarily distributed within the blood stream (30–80 mL/kg) (1–3), smaller IgG-derived competitors, namely etanercept, a dimeric fusion protein consisting of the extracellular ligand-binding protein of p75 TNF receptor linked to human IgG1 Fc, appear to distribute within tissues to a greater extent (0.1 to 0.2 L/kg) (4,5). Since tissue distribution of an anti-TNF agent may lead to greater efficacy in certain autoimmune/inflammatory indications, some next-generation anti-TNF agents have utilized lower molecular weight compounds with greater biodistribution properties to compete against the marketed predecessors (i.e., anti-TNF domain antibody with an apparent volume of distribution of 0.3 to 0.5 L/kg (6)).

Additionally, a diverse set of processes regulates biodistribution of antibodies into tumors as the anatomical and physiological properties of solid tumors are highly different from those observed with normal tissues (7–9). Heterogeneous and leaky tumor vasculature, rapid tumor growth, elevated interstitial fluid pressure (IFP), tumor necrosis, and tissue porosity can introduce challenges for antibody biodistribution into tumors that may be irrelevant to other tissues. Therefore, a thorough understanding of the processes that regulate antibody biodistribution in health and disease will be necessary for the effective application of antibody therapeutics. A unique characteristic of antibody function is the exquisite specificity for the interaction with targeted antigens (soluble or cell-associated); hence, antigen expression and antigen density can impact antibody pharmacokinetics (PK), pharmacodynamics (PD), and biodistribution (10–16). All currently approved intact antibodies are of the IgG class of either IgG$_1$, IgG$_2$, IgG$_4$, or murine IgG$_{2a}$ isotypes. Thus, this article will focus on evaluating factors that regulate IgG antibody biodistribution in normal and tumor tissues.

[1] Preclinical Development (MT) and Corporate Development (HS), AnaptysBio, Inc., 10835 Road to the Cure, Suite 100, San Diego, California 92121, USA.

[2] Cancer Biologics, Astrazeneca R&D Boston, PLC, 35 Gatehouse Drive, Waltham, Massachusetts 02451, USA.

[3] To whom correspondence should be addressed. (e-mail: mtabrizi@anaptysbio.com; mtfard@comcast.net)

OVERVIEW OF FACTORS IMPACTING ANTIBODY BIODISTRIBUTION

Antibody Structure

Each IgG molecule is a large glycoprotein with molecular mass of ~150 kDa containing two identical heavy chains (50 kDa each) and two identical light chains (25 kDa each) linked together by interchain disulfide bonds. Antibody structure has evolved to accommodate the diverse antigen-binding specificities through diversity in the variable region sequence. The antigen-binding site is formed by the intertwining of the light V_L and heavy V_H variable domains. Each V domain contains three short stretches of peptide known as the complementarity determining regions (CDRs); the CDRs are the major determinants of antigen-binding affinity and specificity. The light chain contains one constant domain: C_L. The heavy chain contains three constant domains: C_H1, C_H2, and C_H3. The C_H2 and C_H3 domains allow interactions of the IgG molecule with various components of the immune system by either binding C1q or Fcγ receptors on the immune effector cells. These same variable and constant domains of the molecule can also regulate IgG distribution, elimination, and activity (17–19).

General Biodistribution Mechanisms

Distribution defines the reversible transfer of molecules from one location to another within the body. In general, active and/or passive transport processes govern the movement of molecules across tissue membranes. Active transport requires an energy source, such as a biological carrier, to facilitate the transport of the drug. This carrier-mediated transport can proceed from regions of low concentrations to high concentrations via the action of a receptor or a carrier. In contrast, passive transfer involves simple diffusion of drug and is impacted by factors such as tissue permeability, surface area, concentration, and pressure gradients across the membrane.

Movement of molecules from blood to body tissues depends on various factors as related to (a) drug size, polarity, lipophilicity, and charge, (b) membrane porosity and structure, (c) blood flow characteristics, as well as (d) concentration and pressure gradients. Passive diffusion across membranes depends on factors such as tissue permeability, membrane surface area, and concentration and pressure gradients of drug across the membrane. In general, the rate of distribution may be limited by blood flow (perfusion rate limited) or the permeability of the membrane (permeability rate limited). In the first case, the cell membrane presents no barrier to drug distribution, as it is observed with highly lipophilic drugs, and the rate of transfer is dependent on drug molecules' transit time through the tissue as well as the tissue flow rate characteristics (Fig. 1 a). In contrast, as it is expected with highly polar and charged molecules diffusing across tightly knitted membranes, distribution would be dependent on membrane thickness and physiological structure (Fig. 1 b). Additionally, the process of convection may facilitate transvascular transport of macromolecules. Contrary to diffusion that is proportional to the concentration gradient (Fig. 1 d), convection will depend on the pressure gradient (hydrostatic pressure–osmotic pressure) across vascular and interstitial compartments (Fig. 1 c). The proportionality constant that relates vascular leakage to pressure gradient is referred to as the hydraulic conductivity (20).

For IgG antibodies with similar physical and structural properties (charge, polarity), the transcapillary transport across the blood capillary beds occurs mainly via diffusion and/or convection and, hence, will mainly depend on the capillary endothelium and the underlying basement membrane structure. In general, there are four types of blood capillaries that could facilitate the vascular transport of macromolecules such as IgG monoclonal antibodies (21). First, the continuous capillaries are distributed in structures such as connective tissue, skin, muscle, and a variety of other tissues throughout the body. The continuous capillaries have fully formed membranes, and the endothelial cells form an almost uninterrupted lining rich in tight junctions. The second type, the fenestrated capillaries, is found in the gastrointestinal tract, various glands, and renal glomeruli. The membranes of the fenestrated capillaries contain clefts of about 30 to 80 nm in diameter. Third, the sinusoids have clefts of about 100 nm and are found in organs such as liver, spleen, and tissues like bone marrow. Monoclonal antibodies can freely travel through the sinusoidal clefts. Diffusion of large molecular weight substances such as dextran and IgG (both 150 kDa) in normal tissues was reported using an *in vivo* rabbit model (20,22). Finally, in brain, the capillary endothelium and the underlying basement membrane structure are composed of tight-junction capillaries, and their intracellular junctions are closed by a belt of tight junctions (21,23).

Pharmacokinetic Concepts

The process of distribution can be characterized by a pharmacokinetic parameter known as the volume of distribution (V). As an independent physiological parameter, distribution volume can be influenced by physiological variables such as binding in blood/plasma and tissue, partition into fat, body composition, and body size (24). Volume of distribution of an antibody relates to volume of plasma or blood (V_P), the volume of tissue (V_T), and the tissue-to-plasma partitioning (k_P). Under linear conditions, IgG antibodies are primarily distributed into the plasma compartment and the extravascular fluid following intravascular administration in animals or humans (10,13,14). However, as binding to plasma or tissue targets can influence antibody distribution, it is not surprising that density and expression of the target antigen or, alternatively, active transport processes such as uptake by neonatal Fc receptor (FcRn) may also impact antibody biodistribution.

Role of FcRn in Antibody Pharmacokinetics and Distribution

FcRn functions both to protect IgG from elimination and influence IgG tissue distribution (for a review, see (13)). FcRn is expressed ubiquitously throughout the body in a wide variety of adult tissues. The protective role of FcRn, a major histocompatibility complex class I-related receptor, in regulation of IgG homeostasis was postulated by Brambell *et al.* (25,26). Earlier investigations demonstrated that the clearance rate of IgG was greatly dependent on its serum concentration, whereas the concentration impact on clearance did not apply to other immunoglobulin subclasses such as immunoglobulin M and immunoglobulin A (13,27). Longer IgG half-lives were reported
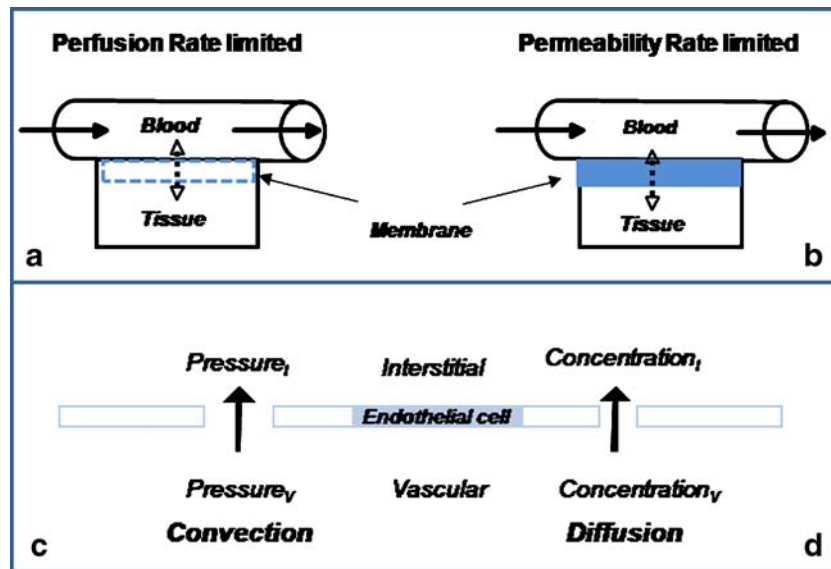
Fig. 1. Movement of molecules from blood to body tissues. In general, the rate of distribution may be limited by blood flow (perfusion-rate limited, *a*) or the membrane permeability (permeability-rate limited, *b*). Mechanisms of transvascular transport: *convection*, due to the pressure gradient (*c*), and *diffusion*, primarily due to the concentration gradient (*d*)

at lower IgG concentrations consistent with Brambell's hypothesis for the protective role of FcRn in IgG homeostasis. The impact of serum IgG concentrations on IgG clearance was also demonstrated in experimental animal models (28,29). Administration of a large dose of purified human IgG (2 g/kg) in mice resulted in a rapid decrease (>60%) in baseline IgG1 and IgG3 mouse serum concentrations (29). However, normal variation in endogenous IgG levels will not impact the elimination rate or distribution of therapeutic antibodies; likewise, the usual therapeutic doses of monoclonal antibodies are not expected to increase total IgG levels to the point that IgG biodistribution and elimination is affected (13). Additionally, the role of FcRn in biodistribution of IgG antibodies was examined recently (30). Lower tissue exposures were observed in FcRn knockout (KO) mice when compared to wild-type animals. However, in the majority of the tissues examined, the ratio of tissue-to-plasma exposure between wild-type and KO animals was not significantly different, suggesting that tissue distribution was primarily due to transvascular transport processes such as convection and/or diffusion (Fig. 1) (30). In some tissues such as skin and muscle, a more prominent role for FcRn in antibody distribution was observed, and in these tissues, exposure profiles did not appear to decline, in parallel with the plasma exposure. This observation is consistent with the structural property of these tissues comprising mainly of a continuous capillary structure with an uninterrupted lining rich in tight junctions—a microenvironment where processes such as convection or diffusion may not be effective due to the larger molecular size of IgG antibodies.

Impact of Affinity on Antibody Elimination and Distribution

Soluble Antigens. In addition to FcRn, three classes of Fc receptors (FcγRs) for IgG interactions have been identified in humans; these receptors are expressed by various phagocytic cells, such as monocytes, macrophages, neutrophils and eosinophils, and other cells of the immune system, including B and T cells, as well as platelets. Human FcγRs bind IgGs with varying degrees of affinity, ranging from low (>10^{-7}M) for FcγRII (CD32), medium (= 10^{-7}M) for FcγRIII (CD16), to high (10^{-8} to 10^{-9}M) for FcγRI (CD64) and can regulate IgG activity and elimination (31–33). Recent findings highlight that Fcγ receptors expressed on macrophages or dendritic cells can trigger the internalization of captured immune complexes, which leads to degradation and the clearance of the antigen–antibody complexes (Fig. 2a). In the simplest case, the *in vivo* steady-state concentrations of endogenous proteins are regulated by their rates of production and elimination. Changes in either antigen production or degradation rates could directly impact the *in vivo* steady-state concentrations of the antigen. Following administration of therapeutic doses of monoclonal antibodies *in vivo*, steady-state concentrations are generally achieved following administration of >4 to 5 doses when dosing frequency is less than or equal to the *in vivo* antibody elimination half-life. Although free concentrations of antigen are suppressed following administration of the antibody doses, a simultaneous increase in the antibody–antigen complex is observed (34,35). The magnitude of the *in vivo* increases in antibody–antigen complex concentrations will be dependent on (a) the turnover rate of the antigen (i.e., antigen synthesis and clearance rates) relative to that observed for the antibody and (b) the elimination rate of the antibody–antigen complex due to interaction with Fcγ receptors (Fig. 2). For example, omalizumab is a humanized IgG1 antibody that inhibits binding of immunoglobulin E (IgE) to its high affinity receptor, FcεRI (36,37). Omalizumab was shown to lower free IgE levels in a dose- and baseline IgE-dependent manner along with simultaneous increases in the antibody-bound complexes (38,39). *In vitro* studies indicated that omalizumab formed a variety of complexes at various antibody/IgE molar ratios (40).
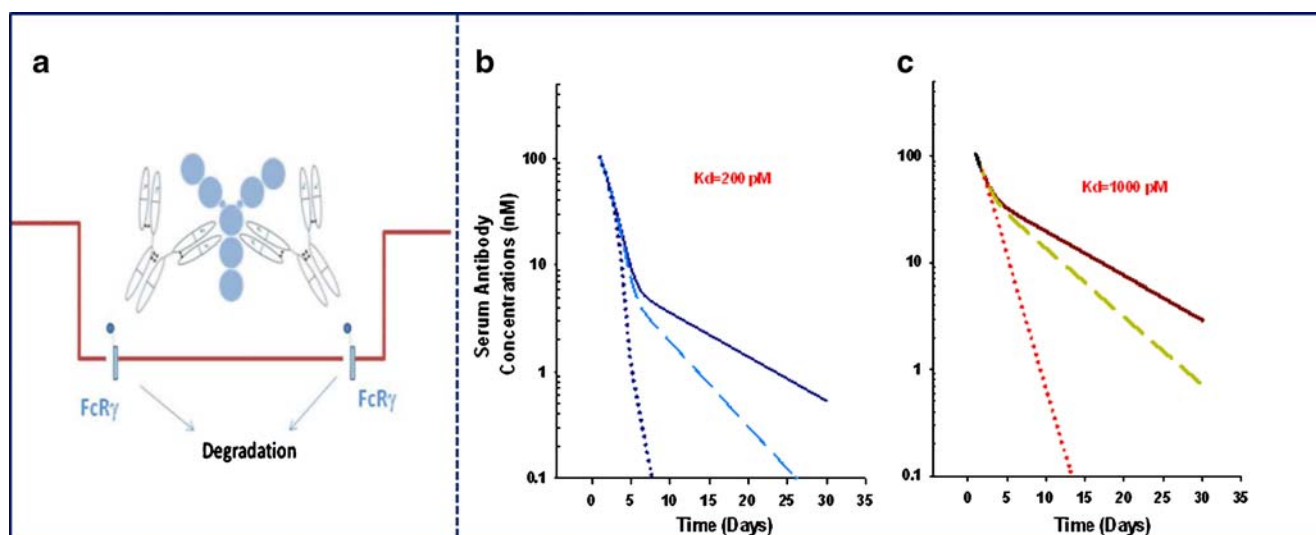
Fig. 2. Fcγ receptor-mediated clearance of antibody–antigen (i.e., omalizumab–IgE) complexes (**a**). Theoretical impact of antibody affinity and antibody–antigen clearance ($CL_{Complex}$) relative to free antibody clearance (*solid line* $CL_{Complex}=CL_{Antibody}$; *dashed line* $CL_{Complex}=x2CL_{Antibody}$; *dotted line* $CL_{Complex}=x10CL_{Antibody}$) on the free antibody serum concentration–time profiles at a simulated affinity of 200 pM (**b**) and 1,000 pM (**c**). Theoretical impact of changes in affinity on antibody exposure was evaluated using a bimolecular interaction PK–PD model. The model accounted for free antibody PK (CL and volume of distribution), bimolecular interactions between antigen and antibody, and the elimination of free antigen. The impact of changes in antibody affinity and clearance of antibody–antigen complex on antibody exposure was evaluated

Indeed, due to target-mediated disposition of omalizumab, as related to formation of large complexes with IgE, nonlinear pharmacokinetics was reported for the free antibody at doses below 0.5 mg/kg (41,42). As formation of these complexes *in vivo* could be impacted by the antibody dose and affinity (more stable complexes can form with higher affinity antibodies), it is possible that increases in antibody–antigen binding in circulation may translate into lower serum and tissue exposure when a more rapid clearance of the complex is anticipated. This hypothesis is supported by previous experimental data demonstrating formation of large immune complexes in mice following simultaneous administration of a therapeutic antibody with its anti-idiotypic counterpart (43). As suggested by theoretical simulations shown in Fig. 2b, when the clearance of the complex is increased by 10-fold relative to the clearance of the free antibody, a proportional decrease in the plasma exposure is predicted. The predicted decrease in serum exposure is more pronounced when antibody affinity is higher for the target antigen (Fig. 2b relative to c). These theoretical simulations project the potential impact of binding affinity on antibody exposure and distribution.

Cell-Associated Antigens. In addition to tumor properties (outlined in next section), penetration of antibodies into tumors can be influenced by factors such as antibody affinity, antigen internalization, and antibody metabolism by the tumor. A number of comprehensive reviews have recently addressed this topic in detail (44–47). Under nonsteady-state conditions, an inverse relationship between antibody affinity and tumor penetration has been predicted (48). This inverse relationship is termed the "binding-site barrier" hypothesis and can be offset by factors such as dose and antibody elimination half-life. For antibody fragments with rapid clearance rates ($t_{1/2} \approx$ minutes for single-chain variable fragment; hours for fragments with antigen binding) and under nonsteady-state

conditions, tumor penetration is predicted to be highly influenced by antibody affinity (45–47). However, full-length antibodies generally have a long elimination half-life and are administered frequently, i.e., weekly, biweekly, or monthly, where steady-state serum concentrations are achieved. Under steady-state conditions, which is a condition generally met in clinical practice, binding equilibrium between antibody and antigen is achieved rapidly, and the movement of antibody through the tumor can be governed by antigen turnover rate, antigen density, as well as the antibody concentration gradient (45–48). In general, cell-associated antigens undergo internalization at constitutive rates, ranging from minutes to days. After binding to cell-associated antigens, antibodies are internalized at a similar rate as the antigen. The internalized complex then is transferred to the lysosome where it undergoes degradation. The newly synthesized antigen following resurfacing can then interact with the unbound antibody. Again under nonsteady-state conditions, it is predicted that tumor exposure can be reduced by the rate of antigen recycling (45–48).

Factors Impacting Tumor Distribution

One of the major challenges in cancer therapy is the hampered ability of large molecules to penetrate tumors efficiently. This limitation is thought to be the result of the architecture and physiology of solid tumors. The poor uptake of antibodies by tumors is the result of slow diffusion rates and the long distances required for diffusional transport in poorly vascularized tumors. This compromised uptake is further exacerbated by virtue of the fact that tumors often lack functional lymphatics (49–52), which can lead to increased levels of IFP (50,53,54). An increase in IFP is likely to reduce convection and thereby inhibit the uptake of antibodies (7,49). Small tumors (or micrometastases), on the other

hand, tend to exhibit a more uniform circulatory system and lower interstitial pressure; as a result, antibody drug delivery is anticipated to be more efficient under these conditions.

Additionally, most tumor blood vessels have an irregular diameter and an abnormal branching pattern. For example, Hori and colleagues have shown that terminal arterioles in rat hepatoma or lung carcinomas approximated 50 branches per 0.1 mm^2, as compared to 14 per 0.1 mm^2 in normal tissues (55). Interestingly, a similar phenomenon occurs in leukemia: irregular and complex branching of microvessels has been observed in leukemic bone marrow, compared with the single, straight microvessels without branching in normal bone marrow (56).

Tumor endothelial cells, although present on most if not all tumor vessels, are actively dividing, unlike the endothelium within normal tissues (57). Tumor blood vessels often do not contain smooth muscle cells, in contrast to normal endothelium, and are characterized by either a discontinuous or absent basement membrane (58–60). Furthermore, tumor endothelial cells also do not form a normal monolayer and therefore do not exhibit normal barrier function. The cells are disorganized and irregularly shaped, and the tumor vessel wall may be lined with only cancer cells or a "mosaic" layer of cancer and endothelial cells (61). These defects render tumor blood vessels leaky (62–64). In fact, studies indicate that tumor vessels are an order of magnitude more leaky than normal vessels. The pore cutoff size (indicating the largest particles that can cross vessels walls) is measured in hundreds of nanometers in tumors, as compared to tens of nanometers for normal endothelium (58,61,65). Also, blood vessels near the center of the tumor are compressed due to the dysregulated growth of tumor cells in a confined space, ultimately impeding blood flow. Pericytes, contractile cells that stabilize vessel walls and participate in the regulation of blood flow, have been reported to exhibit an abnormal association with tumor endothelium and display altered expression of marker proteins (60). While pericytes are present on most tumor vessels, studies indicate that these cells exhibit a loose association with endothelial cells and have cytoplasmic processes that extend deep into the tumor tissue (60). The composition and structure of the extracellular matrix is another contributing factor that can retard the movement of molecules within the tumor (66–68).

As a consequence of the poorly organized vasculature in solid tumors, blood flow is sluggish with unstable rheology. Hence, delivery of oxygen and nutrients to cells that are distant from functional blood vessels is limited, resulting in significant hypoxia and accumulation of products of metabolism, including lactic and carbonic acid, which decrease the pH in this microenvironment (69,70). These oxygen- and nutrient-deprived regions are often necrotic and localized to the central regions of the tumor; as a result of the limited and abnormal blood flow rates in necrotic and seminecrotic regions, efficient drug delivery is severely hindered. For example, the laboratory of Rakesh Jain determined in rodent models that a continuously supplied monoclonal antibody could take several months to reach a uniform concentration in a tumor that measured 1 cm in radius and lacked a blood supply in its center (49,50).

It is also postulated that the aforementioned barriers may hinder the migration of immune effector cells to the tumor (71). In the context of antibody therapy, targeting of malignant cells within a poorly vascularized tumor requires infiltration of immune effector cells and tumor cell killing via Fc receptor-mediated mechanisms such as antibody-dependent cellular cytotoxicity (ADCC). Thus, in addition to poor antibody penetration in this microenvironment, the reduced migration of immune effector cells could adversely impact antibody efficacy.

BIODISTRIBUTION OF MARKETED ANTIBODIES

Antibody Distribution in Lung. Respiratory syncytial virus (RSV) is the main viral respiratory pathogen causing hospitalization in infants and young children worldwide (72). Anti-RSV antibodies have proven highly effective in the prevention of respiratory tract infection and reduction of infant hospitalization caused by this pathogen (72). Palivizumab is the first humanized monoclonal antibody currently marketed in the USA effective in prevention of RSV infection when administered systemically (73). As RSV infection directly impacts the respiratory tract in infants, biodistribution of anti-RSV antibodies to lungs is a critical requirement for pulmonary viral load neutralization following systemic administration (Fig. 3a). Under steady-state serum concentrations, linear distribution of IgG antibodies into lung was reported previously (74). In cynomolgus monkeys, dose-dependent increases in concentrations of mepolizumab, an IgG1 antibody, in bronchoalveolar lavage fluid (BALF) were observed and reported to be approximately between 500- and 1,000-fold less than the steady-state plasma concentrations of the antibody (74). Similar findings have been reported for anti-RSV antibodies (75,76). As improvements in antibody affinity and PK half-life can directly impact viral neutralization as shown in animal models of RSV infection, more potent second-generation anti-RSV antibodies such as motavizumab (MEDI-524) are currently in late-stage clinical development (76,77). Motavizumab is an IgG1 anti-RSV antibody that offers approximately a 20-fold higher *in vitro* potency for viral neutralization as compared to palivizumab (77). Additionally, prophylaxis studies in cotton rats revealed an approximately 5-fold reduction in required serum concentrations (~8 *versus* 40μg/mL) as compared to palivizumab for >2log$_{10}$ decrease in lung viral load (Fig. 3b), the desired clinical endpoint in RSV immune prophylaxis in infants. Following single dose administration of MEDI-524 in monkeys, the partition coefficient for antibody distribution into BALF $\left(k_p = (BALF)^{MED-524}/(Serum)^{MEDI-524} \right)$ on days 4 and 24 postantibody administration were reported to be between 0.001 and 0.002, reflecting approximately >1,000-fold lower effective concentrations (>8 ng/mL) of MEDI-524 in the effect compartment relative to that observed in serum (Fig. 3b). Additionally, improvement in PK half-life via enhancement of FcRn binding for MEDI-524-YTE resulted in further increases in BALF antibody concentrations (75). A 10-fold improvement in FcRn binding resulted in approximately a 4-fold increase in MEDI-524-YTE circulating half-life relative to that observed for MED-524 in monkeys (Fig. 3c). Consistent with the increase in serum exposure, proportional increases in the FcRn-engineered antibody concentrations in BALF were also observed (75). However, when the BALF concentrations were normalized to serum antibody concentrations, no significant difference in antibody concentrations was observed relative to MEDI-524 (75). These results are consistent with previous data reported in
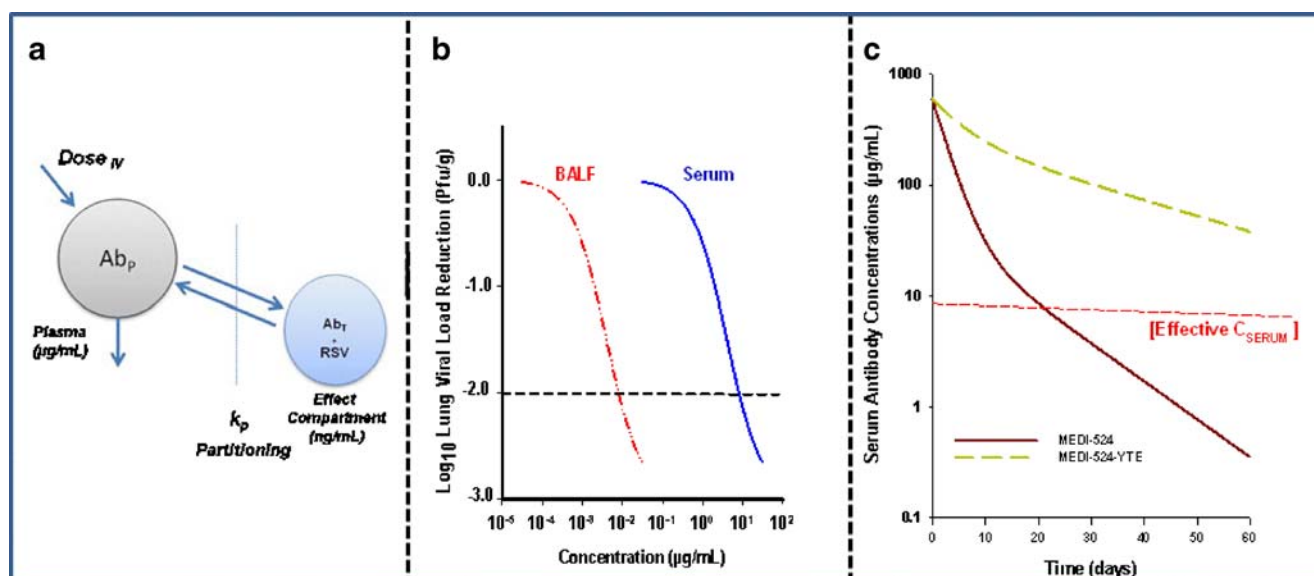
Fig. 3. Biodistribution of anti-RSV antibodies to the lung compartment is a critical requirement for pulmonary viral load neutralization following systemic administration (**a**). Relationships between viral neutralization and MEDI-524 in serum (adapted from (75)) and estimated BALF concentrations assuming a k_P of 0.001 (**b**). Serum concentration–time profiles for MEDI-524 and MEDI-524-YTE following a single-dose administration in monkeys generated from pharmacokinetic parameters estimate reported previously (75) (**c**)

FcRn KO mice highlighting the involvement of transvascular transport processes such as convection and/or diffusion in MEDI-524 distribution into the lung compartment (30).

Antibody Distribution in the Arthritic Joint. Antibodies have been utilized successfully in the management of various inflammatory diseases such as RA, psoriasis, and Crohn's disease. As inhibition of the target antigen in synovium or psoriatic skin is necessary for induction of the pharmacological effect, antibody penetration into these compartments is a critical requirement for therapeutic efficacy. The importance of suppression of cytokines such as TNF, interleukin (IL)-17, and IL-12/23 in synovium during treatment of RA has recently been demonstrated, and the role of the synovial membrane expression of these cytokines as predictive of joint damage and disease progression is now well established (78–82). In line with these observations, effective inhibition of cytokines such as IL-17 and TNF allows rapid control of the inflammatory manifestations of RA and retards cartilage and bone destruction. Previous studies reported approximately >5-fold lower concentrations of IgG antibodies in synovial fluid in human RA patients (83). Due to lower synovial concentrations relative to serum following systemic administration of antibodies, both affinity and pharmacokinetic half-life are among the critical factors that could impact the clinical dose, dosing frequency, and the extent and duration of synovial antigen suppression. This conclusion is supported by a review of

recently marketed anti-TNF antibodies (Table I). Currently, a number of intact IgG antibodies have been successfully used in the management of RA, psoriasis, or Crohn's disease by targeting TNF. Although these antibodies bind to and neutralize TNF, they share few similarities with respect to their affinity, pharmacokinetics, recommended dose, and dosing frequency (Table I) (1,84–87). It is evident that improvements in antibody affinity and pharmacokinetic half-life directly influence the performance of these molecules in the clinic; the most recent anti-TNF antibody marketed in the USA, golimumab, has the highest potency and the longest PK half-life relative to other anti-TNF antibodies with the lowest required effective clinical dose and dosing frequency(Table I) (87).

The theoretical impact of improvements in antibody half-life and affinity on the suppression profile of a circulating antigen can be evaluated using a bimolecular interaction PK–PD model (10–14). The model accounts for antibody PK, bimolecular interactions between antigen and antibody, and the elimination of free antigen. As observed with anti-TNF antibodies, computer projections predict that improvements in antibody PK and affinity favorably impact the antigen suppression profiles in serum and synovium (Fig. 4). As shown, improvements in both antibody half-life and affinity results in more pronounced (40% to 70%) and prolonged antigen suppression following a monthly dosing regimen, i.e., once monthly dosing of a 50- to 60-mg dose *versus* a 40-mg dose administered twice monthly (Fig. 4a). In line with the

Table I. Intact Anti-TNF IgG Antibodies Currently Marketed in the USA for Treatment of Immune-Mediated Inflammatory Disorders

Name (reference no.)	Type	Affinity (pM)	Dose	Dosing frequency	$t_{1/2}$ (days)
Infliximab (86)	Chimeric IgG1	100	3–10 mg/kg	Weeks 0, 2, 6; Every 8 Weeks Thereafter	8 to 9
Adalimumab (1)	Human IgG1	78	40 mg	Every other Week	7 to 17
Golimumab (87)	Human IgG1	4	50 mg	Every 4 Weeks	14
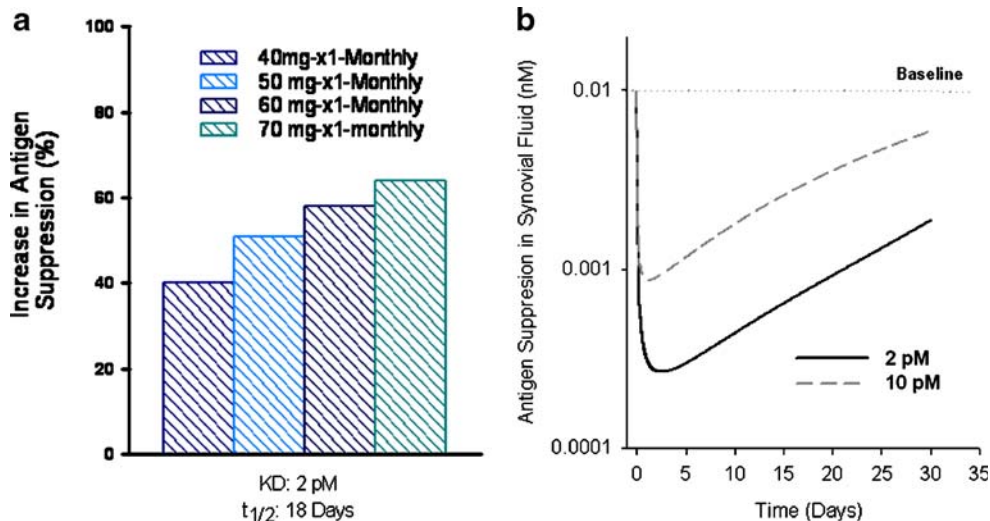
Fig. 4. a Theoretical improvements in area under the antigen suppression curves (*Y* axis) following changes in both antibody half-life (from 12 to 18 days) and affinity (from 10 to 2 pM). These improvements resulted in a more pronounced (40% to 70%) and prolonged antigen suppression following a monthly administration of 40- to 70-mg doses relative to administration of a 40-mg dose twice monthly with antibody affinity of 10 pM and half-life ($t_{1/2}$) of 12 days (not shown). Theoretical impact of improvements in antibody affinity on antigen suppression time profiles in synovial fluid is shown in **b**. Simulations were generated using a bimolecular interaction PK–PD model as described for Fig. 2

lower required dose and less frequent dosing frequency observed with golimumab, the profiles describing the theoretical impact of improvements in antibody affinity on antigen suppression in synovial fluid are also shown in Fig. 4b.

Antibody Distribution in Brain. The capillary endothelium and the underlying basement membrane structure in brain are composed of tight-junction capillaries, and their intracellular junctions are closed by a belt of tight junctions (21,23). Under the conditions where the integrity of blood–brain barrier (BBB) is intact, the presence of tight junctions prevents the transcellular route for diffusion of antibodies across the capillary. In general, antibody penetration into brain (cerebrospinal fluid (CSF)/serum partitioning) has been reported to be >0.1% in animal models and in human patients (88,89). These data are in line with the distribution of endogenous IgG subclasses in normal volunteers ranging from 800- to 1,000-fold less in CSF relative to that reported in serum (Fig. 5) (90). Similarly, these data were reproduced following administration of weekly doses of rituximab in patients with central nervous system (CNS) lymphoma (the antibody in CSF was reproducibly detected at concentrations that were 1,000-fold less than that observed in serum). Therefore, alternative routes of administration, i.e., intrathecal route, were proposed to overcome the poor penetration of rituximab into the brain for treatment of CNS lymphoma (89).

FcRn is shown to be expressed in the CNS endothelium and choroid plexus (91). It is hypothesized that FcRn may play a key role in limiting CNS inflammation in pathological situations such as bacterial infection where IgG can move into the CNS down its sharp concentration gradient (23,92). Under these conditions, FcRn may mediate reverse transcytosis of IgG from the CNS back into circulation. Evidence in support of this hypothesis has been established following direct intracerebral injection of IgG antibodies where IgG molecules rapidly transported back into the circulation via an FcRn-mediated process (93). Indeed, this mechanism has been exploited to reduce amyloid plaque burden in the experimental model of Alzheimer's in rodents (94,95). Recently, the design of a trifunctional fusion construct allowed the application of a new antibody-based therapeutic engineered to cross the BBB in both directions for experimental treatment of Alzheimer's disease (95). The reverse transcytosis of IgG by FcRn may further provide a potential mechanism for therapeutic efficacy of IgG antibodies in the treatment of Alzheimer's disease (see below).

In conditions where the integrity of the BBB is compromised, antibodies have been utilized for treatment of CNS diseases via the systemic route. Recently, bevacizumab in combination with standard chemotherapy was approved for treatment of patients with recurrent glioblastoma (96–98). The exact underlying mechanism(s) for the encouraging responses observed in this patient population is not well understood (97,99). Glioblastoma is a highly vascularized tumor, and anti-angiogenic therapy has proven effective in reduction of vasogenic edema observed in these patients. In the highly vascularized glioblastoma, a dramatic increase in the permeability of BBB blood vessels was observed, and BBB breakdown was reported by the changes of the molecular compositions of both the endothelial tight junctions and the basal lamina structure (100). Similarly, BBB impairment has been identified as a stable characteristic in patients with mild to moderate Alzheimer's disease. For example, the CSF/plasma IgG ratio was shown to be correlated with CSF-albumin index, a marker for evaluation of BBB integrity, in a manner indicating that peripheral IgG had greater access to the CNS in patients with an impaired BBB (101). In line with these data, bapineuzumab, a humanized monoclonal antibody that acts on the nervous system with potential therapeutic value for the treatment of Alzheimer's disease, is currently in late-stage clinical development (102).
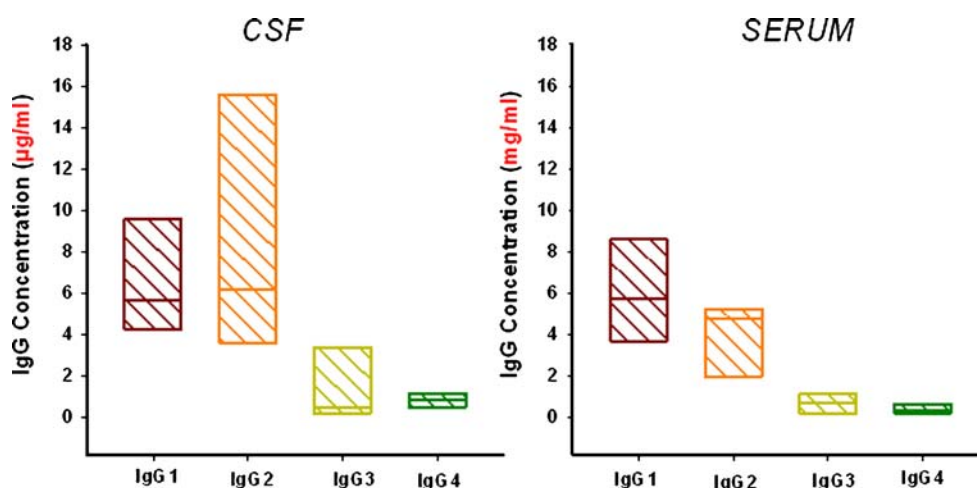
Fig. 5. Concentrations of endogenous IgG subclass in the CSF and sera of normal subjects ($n=7$). The *line in each box* represents the median and the *box* shows the range. Data adapted from (90)

Antibody Distribution in Lymph Nodes, Spleen, and Bone Marrow. Monoclonal antibodies can freely travel through the sinusoidal clefts found in organs such as liver, spleen, and bone marrow. The sinusoids have clefts of approximately 100 nm in diameter allowing antibody movement and biodistribution. For example, rituximab is a chimeric antibody that contains the human IgG1 Fc kappa constant region and murine variable regions reactive with the membrane-associated human CD20 antigen. Rituximab binds to the CD20 antigen found on the surface of normal and malignant B lymphocytes and is currently approved for treatment of relapsed or refractory low-grade or follicular B cell non-Hodgkin's lymphoma (103–105). CD20 is a 35–37-kDa non-glycosylated tetra-spanning membrane protein expressed exclusively by B lymphocytes (104). CD20 is expressed at the late pre-B cell stage and is upregulated and expressed at high levels on most normal and malignant B lineage cells before being downregulated in terminally differentiated plasma cells. Effective distribution and hence depletion of B cells in organs such as lymph nodes, bone marrow, and spleen is a critical requirement for successful clinical application of B cell depleting agents such as anti-CD20 antibodies. *In vivo* binding of rituximab to CD20 positive B cells results in depletion of B cells. Rituximab induces B cell death primarily through complement-dependent lysis and antibody-dependent cellular toxicity effector mechanisms and to a lesser degree via cellular apoptosis (106). Administration of rituximab results in rapid and sustained depletion of circulating and tissue B cells in humans and monkeys (106–109). The strong evidence for biodistribution of rituximab in critical organs such as lymph nodes and bone marrow was recently determined via evaluation of tissue B cell depletion under steady-state antibody serum concentrations (110). Following administration of three weekly doses of rituximab to monkeys where steady-state serum concentrations were achieved, dose-dependent depletion of CD20 positive B cells in spleen, bone marrow, and lymph nodes was observed, underscoring antibody penetration/distribution into these critical organs (Fig. 6). Interestingly, when a second-generation anti-CD20 antibody with enhanced pro-apoptotic activity was administered in monkeys, a more pronounced B cell depletion profile in the same tissues was observed (110). The more efficient B cell depletion observed with the second-generation

anti-CD20 antibody was attributed to improved ADCC and apoptosis induction triggered by this antibody and not due to differences in tissue distribution or immunogenicity (110).

CONCLUSIONS

As the concentration of drug at the proximity of the biological receptor determines the magnitude of the observed pharmacological responses, understanding factors associated
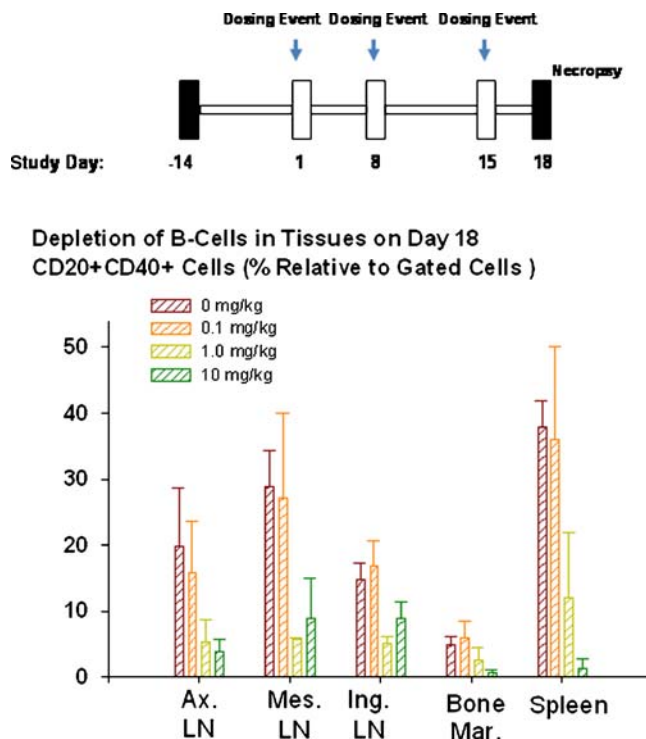

Fig. 6. Dose-dependent depletion of CD20+ B lymphocytes in cynomolgus monkey tissues following administration of three weekly doses of rituximab (tissues were collected and analyzed 3 days after administration of the third dose on day15). The method is described in (110). *Ax. LN.* axillary lymph nodes, *Mes. LN.* mesenteric lymph nodes, *Ing. LN.* inguinal lymph nodes

with antibody biodistribution allows for the intelligent design of therapeutic candidates that colocalize within the intended effect compartment. Improvements in pharmacokinetic half-life via engineering of antibody affinity for FcRn can directly influence the dosing frequency when linear distribution to the effect compartment is anticipated. Additionally, under the conditions where binding is impacted by affinity (i.e., where antigen concentrations are less than or close to K_D), improvements in affinity can greatly impact dose and dosing frequency. As manipulation of antibody biodistribution can particularly be useful in the development of next-generation antibody-based therapies, novel strategies such as multifunctional antibody constructs or antibody fragments and domains should evolve to allow for the smart design of future antibody-based therapeutics.

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