Physiological Barriers to Delivery of Monoclonal Antibodies and Other Macromolecules in Tumors

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Abstract

The efficacy in cancer treatment of monoclonal antibodies or other macromolecules bound to radionuclides, chemotherapeutic agents, toxins, enzymes, growth factors, or effector antibodies has been limited by their inability to reach their target in vivo in adequate quantities. Heterogeneity of tumor-associated antigen expression alone has failed to explain the nonuniform uptake of antibodies. As a result, only in recent years have the peculiarities of tumor physiology been recognized as determinants of antibody distribution. Three physiological barriers responsible for the poor localization of macromolecules in tumors have been identified: (a) heterogeneous blood supply; (b) elevated interstitial pressure; and (c) large transport distances in the interstitium. The first barrier limits the delivery of blood-borne molecules to well-perfused regions of a tumor; the second barrier reduces extravasation of fluid and macromolecules in the high interstitial pressure regions and also leads to an experimentally verifiable, radially outward convection in the tumor periphery which opposes the inward diffusion; and the third barrier increases the time required for slowly moving macromolecules to reach distal regions of a tumor. Binding of antibody to an antigen further lowers the effective diffusion rate of the antibody by reducing the amount of mobile antibody. Due to micro- and macroscopic heterogeneities in tumors, the relative magnitude of each of these barriers would vary from one location to another and from one day to the next in the same tumor and from one tumor to another. If the genetically engineered macromolecules, e.g., lymphokines, killer lymphocytes, as well as low molecular weight cytotoxic agents, are to fulfill their clinical promise, methods must be developed to overcome these physiological barriers. Some of these methods are discussed, and situations wherein these barriers may not be a problem are pointed out.

Introduction

The advent of hybridoma technology and genetic engineering has led to the design and large-scale production of monoclonal antibodies and other biological macromolecules potentially useful for cancer detection and treatment. These molecules can be conjugated to radionuclides, chemotherapeutic agents, toxins, growth factors, enzymes, effector antibodies, or liposomes. Moreover, a number of macromolecular cytolytic molecules (e.g., tumor necrosis factor, lymphokines) and killer cells are under active investigation as potential therapeutic agents. While the concept of using antibodies with a high degree of specificity for tumor-associated antigens remains attractive for cancer therapy, clinical results have not, to date, lived up to the earlier promises of their perceived potential (for review, see Refs. 1 and 2). A key problem with antibodies and other macromolecules is their inability to reach all regions of a tumor in adequate quantities. Heterogeneity of tumor associated antigen expression alone has failed to explain the peculiarities of tumor physiology been recognized as determinants of antibody distribution. Three physiological barriers responsible for the poor localization of macromolecules in tumors have been identified: (a) heterogeneous blood supply; (b) elevated interstitial pressure; and (c) large transport distances in the interstitium. The first barrier limits the delivery of blood-borne molecules to well-perfused regions of a tumor; the second barrier reduces extravasation of fluid and macromolecules in the high interstitial pressure regions and also leads to an experimentally verifiable, radially outward convection in the tumor periphery which opposes the inward diffusion; and the third barrier increases the time required for slowly moving macromolecules to reach distal regions of a tumor. Binding of antibody to an antigen further lowers the effective diffusion rate of the antibody by reducing the amount of mobile antibody. Due to micro- and macroscopic heterogeneities in tumors, the relative magnitude of each of these barriers would vary from one location to another and from one day to the next in the same tumor and from one tumor to another. If the genetically engineered macromolecules, e.g., lymphokines, killer lymphocytes, as well as low molecular weight cytotoxic agents, are to fulfill their clinical promise, methods must be developed to overcome these physiological barriers. Some of these methods are discussed, and situations wherein these barriers may not be a problem are pointed out.

Distribution through Vascular Space

The tumor vasculature consists of (a) vessels recruited from the preexisting network of the host vasculature and (b) vessels resulting from the angiogenic response of host vessels to cancer cells (5, 6). Movement of molecules through the vasculature is governed by the vascular morphology (i.e., the number, length, diameter, and geometrical arrangement of various blood vessels) and the blood flow rate.

Vascular Morphology. Although the tumor vasculature originates from the host vasculature, its organization may be completely different depending upon the tumor type, its growth rate, and its location. The architecture is different not only among various tumor types but also between a spontaneous tumor and its transplants (for review, see Ref. 7).

Macroscopically, the tumor vasculature can be studied in terms of two idealized categories: peripheral and central. In tumors with peripheral vascularization, the centers are usually poorly perfused (Fig. 1). In those with central vascularization, one would expect the opposite. Hence, the penetration of blood borne substances should follow the same pattern. In reality, a tumor may consist of many territories, each exhibiting one or the other of these two types of idealized vascular patterns.

Microscopically, the tumor vasculature is highly heterogeneous and does not conform to the standard normal vascular organization (i.e., artery to arteriole to capillaries to postcapillary venules to venule to vein). Based on their ultrastructure, the tumor vessels can be classified into nine categories: (a) arteries and arterioles; (b) nonfenestrated capillaries; (c) fenestrated capillaries; (d) discontinuous capillaries (sinusoids); (e) blood channels without endothelial lining; (f) capillary sprouts; (g) postcapillary venules (giant capillaries); (h) venules and veins; and (i) arteriovenous anastomoses (shunts) (7). Note that except for vessels of classes e and f, the remaining vessel types are structurally similar to those found in a normal tissue. The vessels of classes e and f are found in healing (granulation) tissue. A key difference between normal and tumor vessels is that the latter are dilated, saccular, and tortuous and may contain tumor cells within the endothelial lining of the vessel wall (7). In addition, unlike a normal tissue with a fixed route between arterial and venous sides, a tumor may have blood flowing from one venule to another via vessels of classes b through g, or directly via an arteriovenous shunt. Furthermore, due to the peculiar nature of the vasculature, the organization

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of vessels may be different from one location to another and from one time to the next. As a result, one would expect different routes for blood flow in the well perfused advancing zone, seminecrotic zone, and necrotic zone (Fig. 1).

Following the pioneering studies of Algire and Chalkley (8), several investigators have measured morphometric parameters of vessels in thin, two-dimensional tumors grown in transparent windows. The pioneering work of Gullino and Grantham (9) led to similar studies in three-dimensional experimental and human tumors (for review, see Ref. 7). The vascular space in tumors varies from 1 to 20% depending upon the tumor type, weight, and method of measurement. Studies in two-dimensional tumors show that vascular volume, length, and surface area increase during the early stages of growth and then decrease; this behavior correlates with the onset of necrosis. The frequency of large diameter vessels increases in the later stages of growth. Most quantitative studies in three-dimensional tumors miss the early growth period of increase in vascular volume, length, and surface area. While studies of later stages of growth show an increase in the intercapillary distance and a decrease in vessel length and surface area, the results on vascular volume are inconclusive. Some studies show that the fractional vascular volume of tumors remains fairly constant during growth (suggesting an increase in the number of blood vessels with sluggish flow), while others show that the fractional vascular volume decreases as a tumor grows (in agreement with the observation that tumor perfusion rate decreases as a tumor grows) (for a review, see Ref. 7). Possible reasons for this discrepancy include errors associated with different measurement techniques as well as presence of arteriovenous shunts and blood vessels with stagnant blood in them. Whether the vascular volume decreases or not, a reduction in vascular surface area would lead to a reduction in the transvascular exchange of molecules. In addition, an increase in the intercapillary distance would require the molecules to traverse longer distances in the interstitium to reach all regions of a tumor.

**Blood Flow Rate.** Most investigators have measured local blood flow rate of tumors based on uptake or clearance of a tracer from a single or a limited number of regions of the tumor. Due to noticeable spatial and temporal heterogeneity in tumor blood supply, these values may not be representative of the whole tumor. A limited number of studies, in which the blood flow rate of the whole tumor has been measured, shows that the average perfusion rate of carcinomas is less than that of the host tissue or the tissue of origin. Sarcomas and lymphomas have higher perfusion rates than carcinomas (for a review, see Ref. 10). In general, as tumors grow larger, they may develop necrotic foci, and as a result, the average perfusion rate decreases with tumor size (10). Note that even in these large necrotic tumors, antibody would be delivered in the well perfused regions.

Since the seminal work of Ide et al. (11), several investigators have examined the microscopic flow heterogeneities of tumors grown in transparent windows. Blood flow in tumor vessels has been found to be intermittent. There are random periods of flow reduction and stasis followed by resumption of flow, sometimes in the opposite direction (12, 13). These fluctuations may result from (a) vasomotor activity of the host arterioles; (b) respiratory or cardiac cycle; (c) passage of RBC, WBC, or cancer cells in a vessel; (d) low perfusion pressures in tumor vessels, and/or (e) elevated interstitial pressure in tumors (for review, see Ref. 7).

Quantitative studies on the macroscopic spatial heterogeneities in the tumor perfusion rate as a function of tumor growth (size) are limited. Based on perfusion rates four regions can be recognized in a tumor: (a) an avascular, necrotic region; (b) a seminecrotic region; (c) a stabilized microcirculation region; and (d) an advancing front. In a rhabdomyosarcoma grown in the transparent chamber in a rat, the widths of the stabilized region and the advancing front were found to remain constant, while the widths of the necrotic and the seminecrotic zones increased with tumor growth. In addition, the perfusion rate in the tumor periphery (i.e., the stabilized and advancing zones) was found to be higher than that in the surrounding normal tissue (12). Intratumor blood flow distributions in spontaneous animal and human tumors are now being investigated using nuclear magnetic resonance and positron emission tomography. While limited, these results are in concert with the transplanted tumor studies: blood flow rates in necrotic/seminecrotic regions of tumors are low, while those in nonnecrotic regions are variable and substantially higher than in surrounding/contralateral host normal tissues (14, 15). As a result of these spatial and temporal heterogeneities in blood supply coupled with variations in the vascular morphology at both macroscopic and microscopic levels, it is not surprising that the spatial distribution of macromolecules in tumors is heterogeneous and the average uptake decreases with an increase in tumor weight.

**Transport across Microvascular Wall**

Once a blood-borne molecule has reached an exchange vessel, its extravasation, \( J_s \) (g/s), occurs by diffusion and convection and, to some extent, by transcytosis. Diffusion is proportional to the surface area, \( S \) (cm\(^2\)), of the exchange vessel and the difference between the plasma and interstitial concentrations (\( C_p - C_i \); g/ml). Convection is proportional to the rate of fluid leakage, \( J_r \) (ml/s), from the vessel. \( J_r \), in turn, is proportional to \( S \) and the difference between the vascular and interstitial hydrostatic pressures (\( p_v - p_i \), mm Hg) minus the difference between the vascular and interstitial osmotic pressures (\( \pi_v - \pi_i \), mm Hg). The proportionality constant which relates transmural diffusive flux to concentration gradients (\( C_p - C_i \)) is...
referred to as the vascular permeability, $P$ (cm/s), and the constant which relates fluid leakage to pressure gradients is referred to as the hydraulic conductivity, $L_p$ (cm/mm Hg s). The effectiveness of the transmural osmotic pressure difference in producing fluid movement across a vessel wall is characterized by the osmotic reflection coefficient, $\sigma$; $\sigma$ is close to 1 for a macromolecule and close to zero for a small molecule (16). Thus, transport of a molecule across normal or tumor vessels is governed by three transport parameters, $P$, $L_p$, and $\sigma$; the surface area for exchange, $S$; and the transvascular concentration and pressure gradients.

Transvascular Transport Parameters. For a macromolecule of specified size, charge, configuration, and binding constants, the transport parameters depend upon the physiological properties of the vessel wall (e.g., wall structure, charge). Ultrastructural studies of animal and human tumors have shown that tumor vessels have wide interendothelial junctions, a large number of fenestrae and transendothelial channels formed by vesicles, and discontinuous or absent basement membrane (16). These characteristics of tumor vessels suggest that they should have relatively high $P$ and $L_p$. As a matter of fact, various tissue uptake studies have found vascular permeability of tumors to be significantly higher than that of skin or muscle (Fig. 2; for a review, see Ref. 16). If tumor vessels are indeed "leakier" to fluid and macromolecules compared to several normal tissues, one would expect reduced transvascular exchange in large tumors compared to smaller tumors (17).2

Transvascular Pressure Gradients. Decreased $p_i$ and/or increased $p_t$ in tumors has been indirectly demonstrated by several investigators working with tumors grown in transparent chambers. By raising venous pressure in the chamber or by loosening the chamber, blood flow can be restored in ischemic/necrotic tumor areas. Direct measurements in sandwich tumors or in the superficial layer of three-dimensional tumors have shown that on the arterial side vascular pressure does not differ significantly between nontumor and tumor vessels, whereas venous pressures may be lower in tumor vessels compared to those in normal vessels (for a review, see Ref. 7).

Since the initial work of Young et al. (19), several investigators have shown that $p_t$ in tumors is significantly higher than in normal tissues (for a review, see Ref. 20). Further, as the tumor grows, $p_t$ rises up to 30 mm Hg, presumably due to the proliferation of tumor cells in a confined space and the absence of functioning lymphatic vessels (5, 20). This increase in $p_t$ also correlates with a reduction in tumor blood flow and the development of necrosis in a growing tumor (20). Investigations of intratumor pressure gradients show that the interstitial pressure is higher in the center of a tumor and it approaches normal physiological pressure towards the periphery [Fig. 3 (20-22)].

In normal tissues $\pi_r$ and $\pi_i$ are approximately 20-25 and 5-15 mm Hg, respectively (17, 18). Although there are no direct measurements of $\pi_i$ in tumors, based on high vascular permeability and high interstitial diffusion coefficient in tumors, one would expect higher concentration of endogenous plasma proteins in the tumor interstitium than in normal interstitium. This hypothesis is supported by the data in the literature (23). As a result, $\pi_i$ in tumors may be higher than that in normal tissues, and may lead to reduced osmotic flow.

As shown in Fig. 3, $p_t$ in tumors is close to zero in the periphery, therefore the filtration of fluid from vessels, $J_f$, would be close to normal. However, as one moves towards the center of the tumor, the increase in $p_t$ would reduce the extravasation of fluid, $J_f$. As stated earlier, convective transport of a macromolecule is proportional to $J_f$; therefore, the rate of extravasation of a blood-borne macromolecule would be negligible in the center of a tumor (17, 18). Since transvascular

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Transport by diffusion is negligible for a macromolecule to begin with, macromolecular extravasation would be very small in the high interstitial pressure regions of a tumor. Since high pressure regions usually coincide with regions of poor perfusion rate and lower vessel surface area, leakage of blood borne macromolecules from vessels would be further restricted.

Transport through Interstitial Space

Once a macromolecule has extravasated, its movement occurs by diffusion and convection through the interstitial space. Diffusion is proportional to the concentration gradient in the interstitium, and convection is proportional to the pressure gradient in the interstitium. The proportionality constant which relates diffusive flux to the concentration gradient is referred to as the interstitial diffusion coefficient, \( D \) (cm\(^2\)/s), and the constant which relates \( u \), to the pressure gradient is referred to as the interstitial hydraulic conductivity, \( K \) (cm\(^2\)/mm Hg-s) (for a review, see Ref. 20). Values of transport coefficients \( D \) and \( K \) are determined by the structure and composition of the interstitial compartment as well as the physicochemical properties of the solute molecule. Larger values of these parameters lead to less hindered movement of fluid and macromolecules through the interstitium. Similarly, large values of interstitial pressure and concentration gradients lead to large convective and diffusive fluxes.

Interstitial Transport Coefficients. The interstitial space in tumors, in general, is very large compared to that in host normal tissues (for a review, see Ref. 20). Similar to normal tissues, the interstitial space of tumors is composed predominantly of a collagen and elastic fiber network. Interdispersed within this cross-linked structure are the interstitial fluid and macromolecular constituents (polysaccharides) which form a hydrophilic gel. While collagen and elastin impart structural integrity to a tissue, the polysaccharides (glycosaminoglycan and proteoglycans) are presumably responsible for the resistance to fluid and macromolecular motion in the interstitium. In several tumors studied to date, collagen content of tumors is higher than that of the host normal tissue. On the other hand, hyaluronate and proteoglycans are, in general, present in lower concentrations in tumors than in the host normal tissue (for a review, see Ref. 20). The lower concentration of these polysaccharide molecules is presumably due to increased activity of lytic enzymes, e.g., hyaluronidase, in the tumor interstitial fluid (for a review, see Ref. 5).

The large interstitial space and low concentrations of polysaccharides suggest that values of \( K \) and \( D \) should be relatively high in tumors. As a matter of fact, the data on hydraulic conductivity of hepatoma 5123 (24) and the data on effective diffusion coefficients of various macromolecules in VX2 carcinoma (Fig. 4) (25, 26) support this hypothesis. An order of magnitude higher values of \( D \) and \( K \) in tumors compared to several normal tissues should favor movement of macromolecules through the interstitium. In several tumors, the necrotic center (7). In such a case, there are no molecules then there is no delivery of macromolecules by blood flow to the necrotic center (7). In such a case, there are no molecules available for extravasation by diffusion across the vessel wall. Also they can reach the center from the periphery (where \( p \), is near zero) by interstitial diffusion. As stated earlier, if the distance between the center and periphery is ~1 mm, it would take days for them to get there and if it is ~1 cm, it would take months (17). If due to elevated \( p \), and cellular proliferation, the central vessels have collapsed completely, if the distance between the center and periphery is ~1 mm, it would take days for them to get there and if it is ~1 cm, it would take months (17). If due to elevated \( p \), and cellular proliferation, the central vessels have collapsed completely, then there is no delivery of macromolecules by blood flow to the necrotic center (7). In such a case, there are no molecules available for extravasation by diffusion across the vessel wall, and consequently the central concentration would be even lower.

Thus far the interstitial movement of molecules which do not bind to any extravascular sites or undergo metabolism has been discussed. It is well known that the binding reaction lowers the apparent diffusion rate of molecules (for a review, see Ref. 27). Therefore, although higher affinity of antibody to antigen significantly increases the concentration of the antibody proximal to the vessel, it retards their movement to distal locations in the interstitium (28, 29). The metabolism of antibodies in normal and tumor tissues is poorly understood. However, the products of metabolism are usually smaller in molecular weight and hence may be cleared relatively rapidly.

Fig. 4. Molecular weight dependence of effective diffusion coefficients, \( D \), of dextran in tumor (T), albumin and IgG in water (W), and IgG in tumor (T), and a nontumor tissue (N). Note that transport is hindered in both tissues compared to water. Despite higher values of \( D \) in tumors compared to nontumor tissues, macromolecules do not reach uniform concentration in a large tumor for a long time due to large diffusion distances.

100 \( \mu \)m distance, ~2 days for 1 mm distance, and ~7–8 months for 1 cm distance (17). These numbers are consistent with the data on the penetration of MAbs in spheroids. Now consider a hypothetical tumor which is uniformly perfused, has nearly zero \( p \),, and has exchange vessels ~200 \( \mu \)m apart. In such a tumor, IgG would reach uniform concentration in ~1 h postinjection provided the plasma concentration remains constant. In a normal tissue with the value of \( D \) lower by an order of magnitude (Fig. 4), it will take ~10 h to reach uniform concentration.

Now consider a more realistic situation, where the tumor vessels are ~200 \( \mu \)m apart and uniformly perfused, but \( p \), has increased in the center so that fluid extravasation, and, hence, convective transport of macromolecules across vessels has stopped. In such a case the only way macromolecules extravasate in the center is by the slow process of diffusion across vessel walls. Also they can reach the center from the periphery (where \( p \), is near zero) by interstitial diffusion. As stated earlier, if the distance between the center and periphery is ~1 mm, it would take days for them to get there and if it is ~1 cm, it would take months (17). If due to elevated \( p \), and cellular proliferation, the central vessels have collapsed completely, then there is no delivery of macromolecules by blood flow to the necrotic center (7). In such a case, there are no molecules available for extravasation by diffusion across the vessel wall, and consequently the central concentration would be even lower.

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Interstitial Fluid Loss from Periphery of the Tumor. It is a well known law of physics that fluid flows from a high to a low pressure region. As discussed earlier, $p_I$ is high in the center of tumors and low in the periphery. Therefore one would expect interstitial fluid motion from the center of a tumor towards its periphery from where it will ooze out into the surrounding normal tissue. Using a tissue isolated tumor preparation, Butler et al. (30) measured this fluid loss to be 0.14–0.22 ml/h/g tissue in four different rat mammary carcinomas. This fluid leakage leads to a radially outward interstitial fluid velocity of 0.1–0.2 $\mu$m/s at the periphery of a 1-cm "tissue isolated" tumor (16). [The radially outward velocity is an order of magnitude lower in a tumor grown in the s.c. tissue or muscle (17).] A macro-molecule at the tumor periphery must overcome this outward convection to penetrate into the tumor by diffusion. The relative contribution of this mechanism of heterogeneous distribution of antibodies in tumors is, however, smaller than the contribution of heterogeneous extravasation due to elevated pressure and necrosis (17, 18).^2

Conclusions, Implications, and Future Perspective

Antibodies linked to radionuclides, drugs, toxins, enzymes, growth factors, and effector antibodies offer a promising approach to the treatment of solid tumors. Their strengths include their high degree of specificity for tumor associated antigens and the fact that exchange vessels and interstitium of tumors are more "leaky" to macromolecules than those of several normal tissues. Their clinical limitation, however, results from their inadequate uptake and non-optimal distribution in tumors. The physiological factors which contribute to the poor delivery in tumors include heterogeneous blood supply, interstitial hypertension, and relatively long transport distances in the interstitium (Table 1). How can these physiological barriers be overcome?

Several physical (e.g., radiation, heat) and chemical (e.g., vasoactive drugs) agents may lead to an increase in tumor blood flow (7, 10, 31). A key problem with this approach is that the increase in blood flow is short-lived and usually confined to well vascularized regions. Increased delivery of macromolecules to well perfused regions would not solve the maldistribution problem.

The second approach may be based on lowering the tumor interstitial pressure. The interstitial hypertension results presumably from interstitial fluid accumulation which, in turn, results from the lack of functioning lymphatics in tumors (5, 17, 20). Since $K$ is a key determinant of interstitial fluid motion, any method which increases $K$ may lower pressure. Use of lytic enzymes (e.g., hyaluronidase) to increase $K$ is one possibility (24). An alternate strategy would be to lower the tumor cell density without destroying the vasculature. Whether fractionated radiation lowers $p_I$ in tumors via this mechanism remains a plausible hypothesis to be tested (16, 17). The use of an osmotic agent (e.g., mannitol) may increase $p_I$ and hence increased antibody penetration (32). However, this increase may be too short-lived to yield practical results.

The third approach may be based on increasing the interstitial transport rate of molecules. Use of cocktails of antibodies would not overcome this problem because each antibody must cross the same physiological barriers. One method of accomplishing this goal would be to use lower molecular weight agents, e.g., antibody fragments $F(ab')_2$ and Fab. While the fragments have higher values of $P$ and $D$ compared to the intact antibody and hence penetrate deeper into tumors, there are two physiological problems associated with their use; they are eliminated more rapidly from blood, and their uptake into normal tissues is also increased. The elimination problem can be overcome by repeated or continuous injections of high doses of nonimmunogenic fragments of chimeric or human antibodies. However, as the molecular weight is lowered further, the normal tissue toxicity problem may become more pronounced similar to that encountered with conventional anticancer agents ($M, < 2,000$) (33, 34). Some of the problems with the systemic toxicity may be overcome by local injection (e.g., intraarterial, interstitial) at the cost of not being able to reach the distant metastases. If the toxicity to normal tissues could be overcome, combination of local and systemic injections would be more effective. Similarly, delivering low molecular weight agents (e.g., drug, toxin, enzyme, hormone) linked to monoclonal antibodies and releasing them once they have extravasated or entered cells seems reasonable. However, once a small molecule is uncoupled from the antibody it may diffuse back into a nearby blood vessel and may be rapidly eliminated. To what extent bifunctional antibodies retard the clearance of small molecules (e.g., individually infused chelated isotopes) from both normal and tumor tissues remains to be tested. Finally, increasing the number of antigenic sites using biological response modifiers such as interferon (35) would increase the concentration of antibody near the blood vessels but would not increase the depth of penetration. One way of overcoming some of these problems is to use radioisotopes with large tumor dose deposition and large depths of penetration; however, toxicity to normal tissues may become a limiting factor. Protecting bone marrow using growth factors (e.g., interleukin 1, colony stimulating factors) or bone marrow transplant may alleviate the normal tissue toxicity problem. Another method is to combine antibody treatment with other modalities (e.g., radiation sensitizers, low molecular weight cytotoxic drugs to synchronize cell cycle) depending upon the tumor type.

In contrast, the physiological barriers discussed in this article may not be a problem for: (a) radioimmunodetection; (b) treatment of leukemia, lymphomas, and small tumors (e.g., micrometastases) in which the interstitial pressure is low and diffusion distances are small; (c) treatment of adequately perfused, low pressure regions of large tumors (36); and (d) treatment with antibodies directed against the tumor endothelial cells or microenvironment of the subendothelial matrix. These barriers may also not pose any problems for treatment with a molecule which has nearly 100% specificity for cancer cells. Until such molecules are developed, methods are urgently needed to overcome these physiological barriers in tumors. It is likely that an improved understanding of tumor physiology will help in developing these strategies. It is also likely that such physiological insight would be a prerequisite to the optimal development of alternative therapeutic strategies for control of established ma-

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<td>Relatively high degree of specificity for tumor associated antigens</td>
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<td>Relatively large vascular permeability, interstitial diffusion coefficient, and hydraulic conductivity</td>
<td>Elevated interstitial pressure</td>
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Table 1 Physiological advantages and problems in the delivery of macromolecules to tumors

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lignancies including the development of anti-angiogenic modalities as well as the delivery of killer lymphocytes (e.g., lymphokine activated killer cells, tumor infiltrating lymphocytes) to the tumor microenvironment.

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References

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