body minus the nine mutations that were introduced by somatic mutation, expressed the protein in *Escherichia coli*, and compared the kinetics of hapten binding with those of the original. The 14,000-fold difference in affinity between the two resulted mostly from a slower dissociation rate of the mutated antibody. This is by far the largest difference attributable to somatic mutation yet seen in a specific antibody, although it has long been clear that substantial gains in affinity of at least 100-fold accompany this progression in the immune system (*7*). This range of affinities is also likely necessary because of the different environments in which cell surface and secreted antibodies operate. The range of affinities for a number of cell surface receptors that bind other cell surface molecules is quite low, from ~10^{-4} M to 10^{-7} M for adhesion molecules and T cell receptors and their ligands (8, 9). Although they are low affinity in absolute terms, these interactions are quite specific and have an effectively higher affinity because of the massive polyvalency and limited (two-dimensional) diffusion characteristic of cell surface interactions. It is reasonable that the germline version of the antibody investigated by Patten *et al.* is lower in affinity, because it should have first encountered the hapten as a multimeric aggregate or on the surface of some auxiliary cell (*10*). Later, as a secreted antibody, the affinity required is generally much higher (in the nanomolar range) and is achieved by somatic mutation. This affinity is typical of many proteins that bind to soluble ligands.

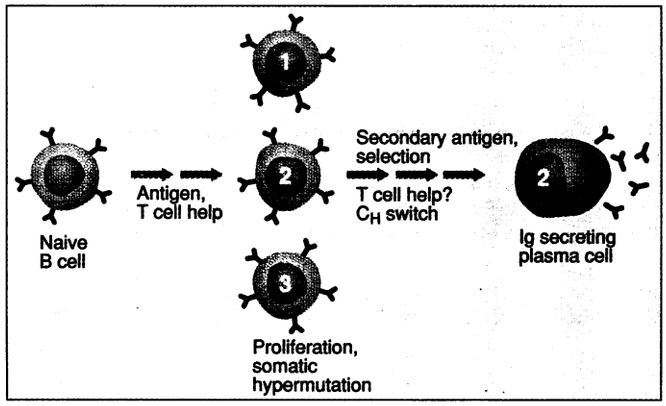

ILLUSTRATION: K. SUTLIFF

Getting stronger: The progression from cell surface to secreted forms of immunoglobulin. Initially, immunoglobulin genes are rearranged and expressed on the surface of B lymphocytes in the IgM form. If they encounter an appropriate antigen and receive T cell help in the form of cytokines, they may somatically hypermutate their rearranged V region genes, and higher affinity variants are selected to secrete antibodies (*6*).

How does somatic mutation generate this higher affinity? Patten and co-workers find that none of the nine somatic mutants are in direct contact with the antigen. Instead, they affect either residues internal to the binding loops [complementarity determining regions (CDRs)] or, in a few cases, are in neighboring residues. This parallels earlier work by Strong *et al.* (5) who saw that a 200-fold increase in affinity between somatic mutants of an antibody to arsonate and its germline predecessor were achieved entirely by mutations in CDR that were not in direct contact with the hapten. Instead, as seen by Patten *et al.*, the most important mutations are involved in inter- or intra-CDR loop interactions. The authors suggest that a unifying mechanism behind this increased affinity could be the stabilization of an optimal binding surface for a particular ligand. [The complex kinetics of some antibody-hapten interactions has suggested that there are multiple binding surface conformations of a given antibody (*11*).]

One variant of this explanation is to consider that affinity is a function of free energy (ΔG) and that free energy is the gain in enthalpy minus the loss in entropy. In this case, mutations that make the antibody binding surface more rigid would decrease the loss of entropy brought about by binding to the antigen and thus increase ΔG and increase the affinity. Alternatively, as discussed by the authors, the changes brought about by the mutations could just be creating a "better" binding site for the hapten. If this were true, however, one would expect at least an occasional mutation in a contact residue, and, although the sample size is small, thus far this has not happened (*2*, *5*).

We now know a little more about both catalytic antibodies and the effects of somatic mutation on antibodies. At the very least, the results of Patten and co-workers suggest that selecting for even higher affinity catalytic antibodies could result in even better catalysts—by further reliance on the immune system during a more lengthy period of immunization or by the more directed approach of mutagenesis with natural or unnatural amino acids.

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Delivery of Molecular Medicine to Solid Tumors

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Approximately one-fourth of all deaths in the United States are due to malignant tumors. More than 85% of these are solid tumors, and approximately half of the patients with these tumors die of their disease. The cause of death is usually metastatic disease distant from the original tumor, although uncontrolled primary (or regional) tumors can also be fatal. The distant metastases are treated systemically with chemical and biological agents, but these attempts are often unsuccessful (*1*).

There is widespread expectation that new strategies, collectively referred to as "molecular medicine," have the potential to be dramatically more effective. The new strategies are a product of the remarkable creativity and energy that has been devoted to molecular biology and biotechnology. The resulting agents include monoclonal antibodies, cytokines, antisense oligonucleotides, gene-targeting vectors, and genetically engineered cells. Because of their potent effect on cancer cells in vitro and in some in vivo tumor systems, these agents have been heralded as breakthrough drugs

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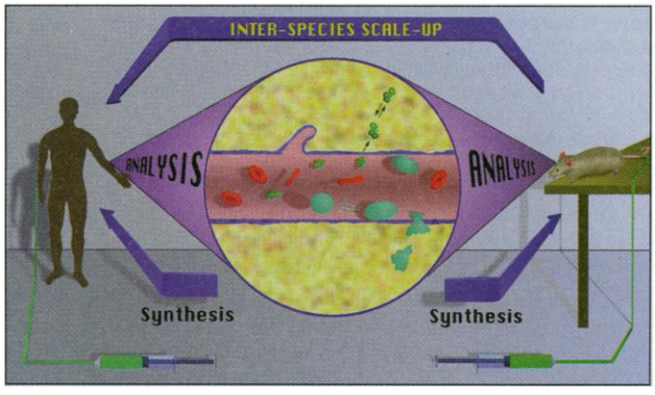
or "magic bullets" and have been enthusiastically accepted as such by policy-makers, investors, and the general public. Although the potential for using these agents in cancer therapy is great and almost certainly justified, clinical results to date have not met the high expectations extrapolated from carefully planned and performed preclinical studies.

No single factor likely explains these disappointing results (*2*). Nevertheless, one problem that needs careful scrutiny is how to overcome the physiological barriers to penetration of drugs into tumor tissue (*3*). For drug therapies to be effective, they must satisfy two requirements: (i) The agent must be effective in the orthotopic in vivo microenvironment of solid tumors. (ii) The agent must reach the target cells in vivo in effective quantities with minimal toxicity to normal tissues. If getting the agent to the target cells is an important criterion of success, what can we do to improve the delivery of molecular medicine to solid tumors? We need a three-pronged strategy.



Delivering molecular medicine. To reach cancer cells in a tumor, a drug (green) must pass into the blood vessels of the tumor, through the vessel wall (dark blue) into the interstitium (yellow), and then migrate through the interstitium. Unfortunately, tumors often develop in ways that hinder each of these steps. We need to better understand the physiological and biochemical barriers in solid tumors, to develop strategies to circumvent these barriers, and to scale up drug distribution from mouse to man. Red blood cell (red); white blood cell (light blue). [Illustration: L. L. Munn]

1) Our current understanding of the molecular genetics, biology, and immunology of cancer is a result of significant investment by federal agencies, such as the National Institutes of Health, and private foundations, such as the American Cancer Society and the Howard Hughes Medical Institute. The advances in molecular and cellular biology have been spectacular and will have immediate benefits for the diagnosis of neoplastic diseases. Unfortunately, efforts to understand the mechanisms of delivery of therapeutic agents in tumor tissue and the limitations imposed by their physiological characteristics have been quite modest. To put things in perspective, let us consider a simple scenario: If we as a society decided to invest all of our money for ground transportation into improving automobiles and virtually no resources in building and maintaining highways and bridges, then at the end of 25 years we would have remarkably efficient cars, but we might not be able to get from one place to another. There are many centers of excellence in the United States for research into molecular genetics, immunology, and other aspects of the basic biology of cancer. However, there are few multidisciplinary teams for the study of delivery of molecular medicine to targets in tissues. The delivery problem is not likely to be resolved unless significant efforts are made in this area.

2) Despite the limitations inherent in animal tumor models, they have provided valuable information for the delivery of therapeutic agents. Molecular genetics will certainly help in the development of more realistic animal models of the human disease (*4*). Even with the best animal model, however, we still need to better understand how the process of biodistribution of various agents "scales-up" from mouse to human. The biochemical and physiological differences between these species make this knowledge critical. The limited mathematical modeling efforts that have been made in this field have resulted in some success for low molecular weight, conventional cytotoxic agents (*5*) and more recently for antibodies and effector cells (*6*). In the chemical and aerospace industries, scale-up from a small pilot plant or an airplane model to a large plant or airplane prototype is now carried out routinely. Success in these fields is a result of extensive research conducted over many decades in fluid and solid mechanics, transport phenomena, chemical kinetics, thermodynamics, and mathematical modeling. Similar extensive and dedicated effort is now needed for extrapolation of drug delivery from animals to humans.

3) The present generation of molecular biologists, cell biologists, geneticists, immunologists, and oncologists is quite familiar with the molecular aspects of neoplastic diseases but has a much more limited knowledge of the integrative pathophysiology of solid tumors (*7*). The excitement over the dramatic advances in basic genetics has understandably generated expectations for the simple application of molecular genetics to complex tissue systems. However, there are no formal programs at present in the United States where scientists and clinicians are educated about the biochemical and physiological barriers to successful cancer treatments. Such training programs are urgently needed.

In today's climate of shrinking federal research support, where would the financial support to achieve these three objectives come from? The results of successful efforts in drug delivery would benefit all pharmaceutical and biotechnology companies producing agents for the detection and treatment of cancer. Therefore, a consortium of companies, along with the federal government, should support these activities. Such a paradigm has been successful in the electronics and chemical industries. Additionally, private foundations, which have supported the revolution in molecular biology in the past, need to realize the central importance of research and training in tumor pathophysiology, so that the large investment in cancer biology will eventually be rewarded.

Leaders in government, academia, and industry must confront the following reality: Soon we will be able to predict an individual's lifetime chances of getting a tumor on the basis of his genetic profile, and we will be able to systematically dissect a tumor to determine which genes are mutated. Will we nevertheless be forced to tell patients that while we have a set of wonderful molecular agents, we cannot deliver them to all target cells in the solid tumors in effective quantities. As the age of molecular medicine and gene therapy dawns, we need to invest extensive effort into uncovering why therapeutic agents that show promise in the laboratory have often been of minimal or no effectiveness in the treatment of common adult solid tumors. It is to be hoped that the three-pronged strategy I have suggested will ultimately ensure that existing and future anticancer agents live up to their tantalizing potential.

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