Pharmaceutical applications of biotechnology: promise and reality

Editorial overview

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Biotechnology — with a heavy emphasis on both 'bio' and 'technology'

Biotechnology has continued to advance the state of the art in pharmaceutical research and discovery, especially in the generation and screening of molecular diversity [1]. Patents have begun to issue on both chemical [2] and biological [3] strategies for creating and evaluating libraries of compounds. Whether structured or random in design, these combinatorial collections, composed of natural and/or unnatural building blocks, are transforming the face of 'lead' discovery and optimization. Surely, the original 'mimotope' strategies of roughly a decade ago will soon yield to true paradigms of molecular evolution. Of course, discovery is only one stage of pharmaceutical R & D, and few groups have adequately addressed important early development parameters such as absorption (for oral delivery), distribution, metabolism, and elimination. Nevertheless, with more compounds having been made and tested in the 1990s than in the combined history of the pharmaceutical industry before 1990, we are witnessing a literal flood of new compounds and biological data not unlike that presenting itself to the various genome projects. In fact, common bioinformatics solutions may pertain to modern combinatorial drug screening and genome data capture, analysis, and database problems.

On to development and commercialization

In the wake of significant disappointments surrounding potential monoclonal antibody (mAb) therapies for septic shock, it is gratifying to recognize the success of interleukins (ILs), interferons (IFNs), and other immunomodulators in certain infectious and inflammatory diseases. While not always without controversy, biotechnology produced enzymes are also proving efficacious: tissue plasminogen activator (tPA; alteplase) for dissolving clots; alglucerase for Gaucher's disease; and dornase α (DNase) for cystic fibrosis. The importance of the immune system in all of these strategies is becoming ever more clear.

If only we could forget

Notwithstanding the history of septic shock, or the difficulties intrinsic to therapeutic approaches for critical care and resulting multiple organ system failure, the future will surely bring more positive results. Russell and Thompson (pp 714–721) summarize the major biotechnology approaches to treating septic shock, discussing the 'yin and yang' of tumor necrosis factor (TNF)-α and IL-1β strategies. The jury is still out on which, if either, will win. Indeed, alternative or combination approaches focusing on different antibodies, tissue factors, antioxidants, complement inhibitors, metabolic mediators, etc may ultimately prove critical to more effective therapeutics.

The promise

Certain biotech products may eventually find greater use outside their original applications. Human growth hormone is an example of a protein with significant off-label use and potential. Aldesleukin IL-2 mutein is an example of a biotechnology product approved for a very specific indication, metastatic renal cell carcinoma, but with potentially far greater utility in infectious disease. Giedlin and Zimmerman (pp 722–726) summarize recent insights into the role of IL-2 and T-helper cells in the control of infectious disease. Schreurs (pp 727–733) extends the discussion to macrophage-inactivating cytokines such as trans-

Abbreviations

BBB—blood–brain barrier; **CAM**—cell-adhesion molecule; **CSF**—colony stimulating factor; **G-CSF**—granulocyte colony stimulating factor; **GM-CSF**—granulocyte/macrophage colony stimulating factor; **IFN**—interferon; **IL**—interleukin; **mAb**—monoclonal antibody; **TNF**—tumor necrosis factor.

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forming growth factor (TGF)-β, IL-10, and cytokines that activate the immune system, including TNF-α and IFN-γ.

Virulence

Recent efforts to identify and characterize virulence factors in pathogenic bacteria using innovative molecular approaches represent an exciting area of research in which basic science has merged with the most modern biotechnology methods [4,5]. Given the importance of this work, and the fact that it is not covered elsewhere in this issue, we comment in more detail on this area in the following section.

Initial work concerning virulence was based on two assumptions: the notion that virulence factors in a particular pathogen are coordinately expressed and, thus, controlled by the same regulatory system; and that virulence factors are often secreted or surface-exposed proteins that interact with host factors in a direct manner. Following the isolation and study of a virulence determinant whose expression can be easily monitored (e.g. a toxin or a hemolysin), it becomes possible to identify other genes or gene products expressed under the same conditions, without knowing much at the start about what these factors might be. This can be accomplished either by assaying total protein profiles in two dimensions after growth under specific *in vitro* or *in vivo* conditions, or by making random gene fusions using transposable elements. The latter method employs a modified transposon to deliver a reporter gene lacking its own regulatory elements. Once the reporter gene has been fused to a gene of interest, reporter expression reflects that of the gene into which it has been transposed.

With the development of the specialized transposon *TnphoA*, which allows investigators to target gene fusions specifically to genes encoding secreted proteins, a convergence of the principles of coordinate regulation and gene fusion has become possible. Thus, Mekalanos and colleagues were able to identify a virulence regulon (comprising more than 15 genes) in *Vibrio cholerae* by using *TnphoA* to identify genes whose expression was coordinately controlled by a single regulatory element. More recently, an elegant refinement of this approach has been the development of random gene fusion technology to screen for promoters that are specifically expressed by microbes during infection. This method is based on the assumption that it might not be possible to identify genes *in vitro* that are expressed, or optimally expressed, only *in vivo*. In this approach, a transposon is engineered with a gene encoding an essential function for *Salmonella typhimurium* growth *in vivo*. The gene *purA* lacks its own promoter but is expressed when transposed downstream from an active promoter. A population of *S. typhimurium purA* mutants, in which transposition of the engineered *purA* gene is allowed to occur, is passaged through mice. Survival of any particular cell is dependent on having *purA* expressed from a promoter active during the course of infection. In this fashion, several genes whose expression is specifically identified during *in vivo* growth can be identified and isolated. This represents an important conceptual advance in our ability to probe the pathophysiology of infectious diseases, and perhaps to identify agents that may intervene at specific and critical steps that are part of the *in vivo* lifecycle of a pathogen.

Back to the future by learning from the past

Growth hormones and colony stimulating factors (CSFs) have a checkered history in the biotechnology industry. They include the remarkable successes of CSFs like filgrastim (G-CSF) and sargramostim (GM-CSF), epoetin α, and growth hormone (somatropin; somatrem), and the less conclusive results (thus far) with growth factors such as the neurotrophins [e.g. nerve growth factor (NGF)]. Ultimately, the pleitropic actions of the many growth factors currently under study will be harnessed to the desired ends, but probably not this year. This is unfortunate, for perhaps the area with greatest untapped potential as a target for biotechnology products is the brain; nonetheless, delivery across the blood–brain barrier (BBB) and specificity to peripheral compartments become major issues biasing many laboratories toward small molecules.

It has been difficult to study and characterize the mechanism for transfer of therapeutic compounds across the BBB. Because of the anatomy of the BBB, the development of cell-based models that relate appropriately to the *in vivo* transfer of drug candidates into the CNS has been problematic. Many CNS drugs cross the BBB via the phospholipid membranes of endothelial cells. To accomplish this task, however, a drug must travel from one aqueous compartment into a lipid membrane and back into another aqueous compartment several times. Osmotic shock can be used to open the BBB, but its non-selective action allows both desired and undesired substances to enter. Of course, active and passive transport mechanisms exist for both small molecules and macromolecules such as proteins. Receptor-mediated uptake (e.g. hormone receptor endocytosis) is a well known phenomenon that has yet to be truly exploited in this respect. Antibody conjugates, polymer encapsulation, and adenovirus vectors represent new approaches to delivery. Reynolds and Weiss (pp 734–738) provide an up-to-date assessment of the untapped potential of growth factors in CNS therapies, with a representative cross-section of this field. While CNS targeting is still more of a promise than a reality, the targeted delivery of immunotoxins, and the like, has much more experience to draw from. Houston (pp 739–744) provides a sobering look at the the experience, to date, in cancer (among other diseases) and reviews the problems and opportunities.

Last, but certainly not least

Inflammatory processes are currently implicated in arthritis, cancer, infectious disease, Alzheimer's disease, and everything in between. The immunology community has finally hit on the hottest of targets — cell-adhesion molecules (CAMs). The explosion of publications and patents in the CAM arena is staggering. These multiple gene families (integrins, selectins, etc) provide a wealth of new targets for immunologists and drug-hunters alike. Peltz (pp 745–750) gives a fresh perspective on this burgeoning field.

Adolescence

Maturation of the biotechnology industry has led to the recognition that only some products, when targeted to the appropriate patient population with convincing efficacy, can reach marketplace with due speed. Other products have taken, or will take, the more traditional pharmaceutical course of a decade or more, with R & D expenses in excess of $100 million. Perseverance in the biotechnology industry has, at times, spanned several companies and multiple partnerships. Fortunately, biotechnology patents have proven more protective than those for more classical pharmaceuticals. Historically, 30–40 new therapeutics of all classes were introduced for the first time in 1990, 1991, and 1992. Nearly 10% of these introductions were biotechnology products, notably, several IFNs and CSFs, and alglucerase (a modified glucocerebrosidase). Of course, with the typical flurry of approvals at the year's end, we don't yet know how many new human therapeutics will be introduced for the first time in 1993. But, from a biotechnology perspective, some potential blockbusters are on the horizon. These include IFNβ-1b (introduced into the marketplace this Fall) for multiple sclerosis and DNase for cystic fibrosis and chronic bronchitis. The setbacks for IL-1 receptor antagonist (IL-1ra) and sepsis mAbs like HA-1A and E5 are also noteworthy, if not entirely unprecedented.

The reality

Despite the cloud of 'Clintonomics' currently hanging over the US pharmaceutical industry, and the general trend toward critically introspective value-based pharmacoeconomics worldwide, the combination of biotechnological and pharmaceutical technologies and strategies should allow key players not only to remain strong, but also to enhance their competitive positions. Whereas, in the past, solid development skills alone enabled pharmaceutical companies to succeed profitably, in this decade, both creative research and innovative development programs seem the requirement for success. To date, one might conclude that the promise of biotechnology has already delivered a desirable reality. In 1992, about 10 biotechnology products were listed in the top 100 pharmaceutical products (Med Ad News, 'The Leading 100 Drugs Worldwide', May 1993, ppS5). Enzymes like DNase, perhaps, will join this meritorious group in the near future, and new applications (e.g. multiple sclerosis) for IFNs will expand the marketplace for biotechnology derived therapeutics. Moreover, with a much smaller infrastructure to support, each biotechnology product contributes more to the well being of its progenitors than an equivalent pharmaceutical. More than ever before, biotechnology products can claim their rightful position alongside pharmaceuticals. Surely, the past promises of biotechnology are simply a prologue and the future realities sublime. Stay tuned!

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