Animal Cell Biotechnology

Volume 4

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Preface

The Animal Cell Biotechnology series of books seeks to provide its readership with a complete and in-depth compendium of knowledge of both the theoretical and practical aspects of the subject. To achieve this end the editors have adopted an approach that allows them to focus on a particular aspect or topic area in each volume while at the same time making space available to bring readers up to date with those special areas where the editors judge that the rate of progress is such that the new information needs to be highlighted for special attention. In this way the series will reflect the way in which the subject is advancing as well as providing the background information. Thus investigators who are moving into the area from other activities will be able to both access and appreciate in some depth the wealth of information, which is already available, as well as coming to grips with facets that are dynamically progressing.

The present volume has for its focused topic the second generation of bioproducts which can be derived from animal cells in culture. While the first generation may be designated as the live and killed whole virus vaccines the second generation is clearly led by the monoclonal antibodies and lymphoblastoid interferon. This volume reflects these developments in the chapters dealing with therapeutic, non-therapeutic, chimaeric and anti-idiotype aspects of monoclonal antibodies and interferon. Interferon produced by animal cells in large-scale suspension cultures has vied for the dominant position with hybridoma systems. While culture systems using hybridomas are in widespread use in many small-, medium-sized and large companies in most of the countries of the developed world, and some of the undeveloped world, the lymphoblastoid alpha-interferon culture system is in production in fewer countries although the scale of operation is generally larger than that used for monoclonal antibodies. Furthermore, as a result of the painstaking work carried out, for the most part, at the Wellcome Foundation, U.K., it has been possible to obtain regulatory agency approval for the use of the alpha-lymphoblastoid interferon, which has been derived from an overt cancer cell

for use in humans. This major breakthrough in the licensing of a product of a transformed cell has reverberated round the industry so that development of many new products based on expression systems using BHK or CHO cells are now well under way.

As an indicator of a product area which is rapidly developing, the chapter on the production and use of the colon cancer antigen typifies the way in which molecules made by animal cells can be of importance in diagnostics and therapeutics. Other such chapters can be expected based on the exploitation of receptor molecules (CD_4 receptors for HIV, for example), or molecules of the extracellular matrix. Hormones and cytokines represent a class of biochemicals which are becoming an increasingly important part of the armament of the practising physician. The chapter on erythropoietin represents the development.

In the rapidly moving areas of endeavour the increase in the knowledge base and the technical skills relating to our abilities to move genes into animal cells and have them expressed in such a way as to make products economically viable has been particularly impressive. This on-going activity has not yet reached its apogee and the chapter which relates these events sets the scene from which future advances are sure to arise. The alleged fragility of animal cells to hydrodynamic shear and bubble aeration also command attention. Chapters on these subjects seek to apply rational approaches to determine and explain the phenomena which have led to this "mythical" contention. There is little doubt that many failed experiments have been attributed to the defenceless nature of the delicate-cell-wall-less animal cells. It appears more likely that a strained physiology plays a much more important role in such failures than the apparent anatomical inadequacies. Rapid advances characterize the area of immobilized cell cultures. Such systems have local cell concentrations approaching those which prevail in animal tissues (1E9 cells/ml), in volumes of 0.1 ml to a questionable 200 ml. Many new systems based on fluidized static particles or on bundles of hollow fibres are present in the market place. While such systems have to be reconciled to a clear leader of the technique there is much activity which is dealt with in the chapter on this subject.

The HIV has commanded the attention of the scientific community and the public alike. Our knowledge base is unparalleled in its depth, detail and scope yet our ineffectiveness in alleviating the suffering already caused by this virus or the personal and social pain to come has put those of us who are attempting to engineer solutions to this desolator in difficulty. The chapter in this volume on this topic deals with the production of the virus. This key step will surely enable us to come to terms with the challenge presented by the virus and will be instrumental in eventually overcoming the threat it poses to humanity.

Finally in an overview of the contemporary issues in the area of animal cell biotechnology the challenge of transgenic animals is reviewed and found wanting while the dogma of using large molecules derived from animal cells to make small molecule pharmaceuticals is assessed and found to be ahead of its time.

R. E. Spier
J. B.-Griffiths

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1

Introduction to Volume 4: Contemporary Issues in Animal Cell Biotechnology

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1. BIOPRODUCTS FROM ANIMAL CELLS IN CULTURE

Projections for the new biotechnology industry, (DNA manipulation/hybridoma/transgenics), suggest that the market for its products could amount to $50 billion per annum by the mid to late 1990s. It has also been conjectured that some 40–50% of that market could be for products derived

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from animal cells in culture (*1*). To some extent this can be supported by the figures available to date (*2*), in which high value animal-cell-derived products such as tissue plasminogen activator, interferons and monoclonal antibodies which improve kidney grafting procedures, are estimated to account for about 50% of the market. The market for other monoclonal antibodies, cytokines, enzymes, immunoprophylactics and hormones derived from animal cells is set to increase considerably and any listing of products in clinical trials attests to the major impact that animal cell biotechnology will have on the pharmaceutical industries of tomorrow (*3*).

It is also certain that animal cell cultures will move into areas which were once the reserve of whole animals in toxicity and potency testing of materials which are to be injected into, or laid onto, human and animal bodies. This transformation is already in progress (*4*), but it is as yet a relatively undeveloped field from the point of realization of its economic value. That such prospects are firmly based in realism may be evidenced by the growing number of companies who have made it their business to supply the needs of the animal cell biotechnological community with the firmware (medium, serum, growth factors, vectors, assay reagents, cell culture substrates), hardware (bioreactors, probes, automatic analysis equipment, clean air cabinets) and software in the form of a burgeoning literature; to which this volume is contributory. It is to be remembered that 10 years ago there was barely a single work specifically devoted to animal cell biotechnology.

2. THE CHALLENGES AHEAD

Unlike the past, when the animal cell culturist only faced the challenge of the genetically engineered prokaryote and lower eukaryote there has recently emerged a new threat from the transgenic animal and/or plant which can be made to make products which would be similar to, though hardly ever identical with, materials made by animal cells in the normal body. With regard to the traditional challenge, it is now well-recognized that animal proteins made in engineered bacteria and yeast are not post-translationally modified in a manner identical with that which occurs within the animal cell (*5*). However in spite of such a limitation, producers of animal-originating biomolecules persist in using such materials as the manufacturing process is cheaper. This situation may survive in the short term but improvements resulting in considerable cost reductions in the unit price of products derived from animal cells in culture are in hand and should significantly modify this picture in the longer term.

Other issues, which are not less challenging than those referred to above, are those which seek to make animal cell biotechnology into an activity which

is more dependent on science and theory than art, craft, skill or practice. Contingent with this thrust is the need to achieve a higher order of standardizability with respect to the quality and performance of both the cell substrate and the medium whereby it is nurtured. This latter facet could be accomplished were the activity as a whole weaned from its dependence on animal sera, irrespective of whether such sera are of the rare and expensive variety (foetal calf serum) or the more mundane adult bovine serum.

2.1. Transgenic Animals

With respect to the transgenic animals (the transgenic plant production systems are likely to run into the same problems experienced with the prokaryotes and lower eukaryotes), there are a number of reasons which suggest that this route to animal protein bioproducts is not without its problems (6). While recognizing the inherent advantages of making a bioproduct of therapeutic value in place of, say, the lactalbumin of milk, the ovalbumin of eggs or the serum of blood, it is as well to ponder the contraindications.

1. So far the yield of the expressed protein from the inserted gene has delivered poor yields of product materials, although active protein has been detected with such examples as tissue plasminogen activator and growth hormone. (The latter system is of more interest in increasing the yield and efficiency of production of whole animal protein than in providing growth hormone as a human therapeutic product.)
2. As most of the biologicals which are derived from animal proteins are complex in nature, the regulatory agencies require that the product be defined as much by the process of its production as by its composition and properties. It is therefore necessary to define the animal from which the material arises. Clearly this is much more difficult to do for a whole animal than for a stainless-steel bioreactor.
3. Part of the process of source of product definition is the ability to determine unequivocally the state of septicity of the producing system. Recent experience with endogenous viruses or disease-causing infectious agents (retroviruses and prions), and the present concerns of the regulatory agencies for the viruses which we have not yet identified or even become aware of, will present severe problems in the acquisition of product licences (7). These problems are exacerbated when whole animals are used as the producing substrate and are barely containable when well-characterized cell lines are the source of the bioproduct. In the latter case there is a wealth of historical information which allows