edited by D. F. METTRICK
and S. S. DESSER

PARASITES—THEIR WORLD AND OURS

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Proceedings of the Fifth International Congress of Parasitology held in Toronto, Canada, on 7-14 August, 1982. Under the auspices of The World Federation of Parasitologists.

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PREFACE

It is 18 years since the First Congress of Parasitology was held in Rome in 1964. Since then, successive congresses have attempted the difficult task of creating that ideal mix of environment, speakers, and a social programme that results in an outstanding congress. As we write this introduction to the proceedings of the ICOPA V we hope that it will indeed turn out to be a most successful meeting.

In the 18 years since ICOPA I, the field of parasitology, like other areas of science, has continued to become more specialized, as seen by the formation of new societies interested in specific aspects of parasitology, and in the publication of new specialist journals, of which molecular and biochemical parasitology, systematic parasitology, and parasite immunology are recent examples. Similarly, the Commonwealth Agricultural Bureaux now publish separate abstract journals for animal and human helminthology, plant nematology and protozoology.

As parasitology continues to fragment and become more interdisciplinary, so the importance of ICOPA becomes greater, by providing an increasingly unique opportunity for clinicians, veterinarians, epidemiologists, immunologists, ecologists, economists, and many other specialists, to get a broader perspective of the whole picture as it relates to the importance of diseases and losses attributable to parasitic infections around the world.

Our first task, therefore, in planning ICOPA V was to select 10 major fields covering the breadth of parasitology, and then to invite 30 speakers to address the difficult task of reviewing new findings in each of the major fields of our discipline. These plenary reviews will provide both the expert and the general parasitologist with a critical summary of recent advances.

For those readers and participants who wish to be brought up to date in more narrow specialist fields, we also invited a further 90 speakers to present a review of their own area of expertise. These speakers, which we termed "discipline lecturers", were selected in consultation with the appropriate plenary lecturers, and the sessions were planned so that the subjects covered were inter-related.

The complete text of the plenary lectures and 3-4 page summaries of the discipline reviews, which are the subject of this volume, therefore provide an overview of the state of parasitology in 1982. For further detailed information readers are referred to the companion volume, "Abstracts of Papers presented at ICOPA V", which reports on the most recent laboratory and field research. Together, they represent a significant contribution to our discipline.

Recognizing that the expected 2000 participants represent only a fraction of the world-wide interest in parasitology we have arranged to have this volume published concurrently with the congress. Participants will take their copies home with them, and those who unfortunately could not come to Toronto, will be able to purchase these proceedings immediately.

We hope that this rapid publication will greatly facilitate the dissemination of new information and that it will act as a stimulus to our research. The proceedings will also allow the incorporation of the latest advances into our lectures and tutorials.

Finally, we are pleased that so many scientists and accompanying persons from more than 80 countries were able to come to Toronto and participate in ICOPA V.

David F. Mettrick
Sherwin S. Desser

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NEW TECHNOLOGIES FOR PARASITOLOGY

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INTRODUCTION

How new are the Monoclonal Antibody and Recombinant DNA technologies? The potential value of specific monoclonal antibody-secreting hybrid cell lines derived from fusion of spleen cells from immune donor mice with myeloma cell lines was first described in 1975 by Kohler & Milstein (1). It is rather more difficult to identify a key starting point for recombinant DNA technology. As we know it today, gene cloning has become possible only through a series of major developments in techniques for the manipulation and characterisation of DNA. If any single event provided the key to gene cloning it was the isolation, in 1970, of the first sequence-specific DNA-cutting enzyme (restriction endonuclease). This led, in 1972-73, to the first precise construction of recombinant DNA molecules *in vitro* and their insertion into bacteria. The years 1973-1976 were clouded by the great safety debate (2) but thereafter the pace of research, discovery and publication in this field has grown exponentially. The first company to be founded exclusively for the commercial exploitation of Recombinant DNA techniques, Genentech, was established in 1977 and a Patent application, for the bacterial synthesis of Human Insulin A and B chains, was filed in the same year. Safety considerations, with emphasis on biological containment of Recombinant DNA molecules, led to the development of the bacteria, plasmid and bacteriophage strains which are now in everyday use. Although *Escherichia coli* remains the workhorse of gene cloning, other bacterial, mammalian and yeast host-vector systems are now available for the selection and expression of Recombinant DNA. Other key developments have occurred in gene synthesis and in DNA sequencing. More than 80 restriction enzymes with over 30 distinct sequence specificities have now been characterized and it is the widespread availability of these and other enzymes and techniques, together with the dissemination of the relevant expertise, which has made gene cloning the universal and powerful technique as we know it today.

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MONOCLONAL ANTIBODIES

The crucial experiment which generated the first monoclonals (1) was an extension of studies on the fusion of mutant myeloma cell lines which were initiated with a view to extending the current understanding of the regulation of immunoglobulin gene expression. The original fusions used Sendai virus and were very inefficient, not least because of the apparent lack of Sendai receptors on mouse myeloma cells (3). Polyethylene glycol is now universally used as a fusion-promoting agent (4).

Three types of myeloma cell lines are in general use for the generation of hybridomas. Least used is the original Kohler-Milstein P3/X63 which has the disadvantage of producing indigenous immunoglobulin whose light and heavy chains can also contribute to the formation of all possible hybrids with the spleen cell derived L and H chains, thus producing a complex spectrum of immunoglobulin molecules such that only a small percentage will have the desired specificity. This disadvantage is reduced by using the related NSI cell line which is unable to synthesize H-chains (5). Despite the more recent availability of lines which produce neither H or L chains, NSI has been the myeloma line most widely used for the generation of mouse hybridomas. Although, in principle, hybrid immunoglobulins containing the parental myeloma k L chain can be produced, this does not always happen in practice. Rat and human hybridoma systems have also been described. Potential advantages of the rat system include the possibility of differing mouse and rat responses to some antigens and a larger sized animal for more efficient serum and ascitic fluid production. The main potential for human hybridomas may lie in the production of therapeutic antibodies but, with the yields of immunoglobulin which can presently be obtained from cell culture, it seems likely to be some time before this objective will be economically viable. Gene cloning may play a role in developing this approach.

The potential uses of monoclonal antibodies are limitless. They may be used in a wide range of serological tests where they have advantages over conventional antisera. They have found widespread use in defining cell populations and for exploring cellular structures by immunocytochemical techniques. A recent publication (6) shows the advantages for high resolution radioimmunocytochemistry in being able to intrinsically label monoclonals produced in tissue culture.

The advantages of monoclonals derive from three basic properties of the technique: first, monoclonal antibodies can have exquisite specificity; secondly, that specificity can be obtained without the need for extensive

prior purification of an antigen; thirdly, the monoclonal product is a defined chemical with constant properties. Monoclonals of different classes and subclasses and of differing affinities and with specificities for different epitopes on the same antigen may be produced. In the event that the specificity of a monoclonal antibody itself might be too narrow, the monoclonal can be advantageously employed in the purification of an antigen which can then be used to produce a highly specific polyclonal antiserum.

In principle, the techniques for producing monoclonal antibodies are simple and have been well described in the literature (for experimental techniques and general discussion see references 3,7). They are very labor-intensive and require meticulous cell culture technique. The primary drawback derives from the random nature of the essential events involved in the practical realisation of the conceived qualities of the antibody which is being sought in each situation. As with most biological techniques, there is no substitute for experience and the best course of action is to take advice from successful exponents of the techniques. If you are starting from scratch, be sure to get your myeloma cells from a reliable authenticated source. They must be growing well to fuse well. Given the relative inefficiency in generating hybrids (about $1/10^5$ spleen cells), it is important to maximise antibody production by the donor spleens. Immunization may be achieved in one of several ways, ranging from injection of purified or enriched antigens, disrupted cell homogenates, fixed or irradiated cells, or live infections which can generate natural immunity. Most fusions have been performed after one or a few immunizations using different protocols, with or without adjuvants as appropriate, but almost all involve a final intravenous injection of antigen 3-5 days prior to fusion. Antibody titer should be determined prior to fusion and spleens from the highest titer mice should be selected for fusion.

In many of the successful fusions performed by my colleagues, several hundred antibody-positive culture wells have been found in the initial screening of hybrids. As the labor involved intensifies as cultures are expanded, cloned and screened, only 10-20 positive wells can generally be selected from the initial screening. It is therefore most important to use appropriate screening techniques at this early stage of hybridoma selection. Unfortunately, on average, 50% of the initially selected hybrids will be lost due to instability, poor growth or cessation of secretion during subsequent handling up to and including growth as ascitic tumors.

The method chosen to screen for hybrids should reflect the properties required of the desired hybrid. This may seem obvious but it is frequently