GUEST EDITORIAL

HYBRIDOMAS – MEDICAL DIAGNOSIS OF THE FUTURE

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INTRODUCTION

ANTISERA prepared in animals or obtained from human sources and containing specific antibodies against a particular antigen are widely used in medical diagnosis. For example, these preparations are used in:

1. diagnosis of various microbial infections e.g. detection of hepatitis B surface antigen (HBsAg) and final identification of bacterial organisms isolated from clinical specimens.
2. quantitation and determination of plasma proteins e.g. immunoglobulins and complement.
3. detection of cell surface antigens e.g. blood grouping (ABO and Rh antigens), HLA typing, enumeration of B lymphocytes.
4. immunoflurorescence tests e.g. detection of *auto*antibodies using tissue sections; rapid diagnosis of viral infections on clinical specimens.
5. radioimmunoassay procedures to measure levels of various hormones (e.g. insulin, T3, T4, TSH), IgE, alpha-foetoprotein (AFP) etc.
6. pregnancy testing ie. the use of anti-HCG antisera.

Several problems, however, are associated with the production and use of these antisera preparations. Immunization of an animal with an antigen produces *many different antibody molecules to the same antigen.* Hence, antisera from these animals invariably represent a complex mixture of antibodies of *polyclonal* origin which are also heterogenous with respect to affinity and specificity towards the immunizing antigen. Additionally, two or more different animals never produce both quantitatively and qualitatively the same immune response (ie. the same antisera) hence giving rise to problems of continuous reproducibility and standardization.

Ideally, antibodies used in medical diagnosis should be monospecific and homogenuous ie. *monoclonal* in origin. This goal, as mentioned above, is very difficult to achieve by immunizing animals and obtaining their sera. However, a recent development in cellular immunology has provided an elegant solution for the *in vitro* production of monoclonal antibodies of a desired specificity for unlimited time and theoretically, at least, in unlimited amounts. The technique was developed by Kohler and Milstein (Kohler and Milstein, 1975, 1976; Kohler *et al.*, 1976) who fused normal antibody-producing cells (B lymphocytes/plasma cells) with an appropriate B cell tumour *in vitro.* These tumour cells (myeloma cells) are 'immortal' and can be grown indefinitely in *in vitro* culture. The fusion results in a *hybridoma* ie. a hybrid cell line originating from a single antibody-producing cell which is immortal and which is also producing antibodies to a specific antigen. Cloning of these cells gives rise to a uniform line of cells producing absolutely identical (ie. monoclonal) antibodies. With these type of antibodies all problems of standardization of medical diagnostic reagents disappear and the antibodies produced are of unprecedented specificity thus also eliminating the problem of cross-reactions with other antigens (New Scientist, 1977; Staehelin, 1978).

THE HYBRIDOMA TECHNOLOGY

The *in vitro* fusion between myeloma cells and

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antibody-producing cells (normally spleen cells from an immunized animal) is achieved by treating the cell mixture with inactivated Sendai virus (Kohler and Milstein, 1976) or, more commonly, with polyethylene glycol (Pontecorvo, 1975). The stimulated spleen cells (plasma cells) produce antibodies against the desired antigen but are themselves unable to survive and proliferate in tissue culture medium. The immortality *in vitro* is provided by the fused myeloma cell. However, in order to select out the myeloma cell which has fused to spleen cells from the many unfused myeloma cells, one has to use a *mutant* myeloma which cannot survive in a special selection medium unless it is fused with a normal cell (ie. the spleen cell) which cures the genetic defect. This defect is usually in the form of a missing enzyme now supplied by the spleen cell. Thus, only the hybrids will be able to survive and proliferate in the growth medium (unfused spleen cells eventually die because they cannot divide *in vitro*] (Kohler and Milstein, 1975, 1976). Following fusion, the cells are *cloned* and after further growth, the antibody product of these hybrids can be detected in the culture supernatant by a variety of methods including radioimmunoassay, ELISA (enzyme-linked immunosorbent assay) and indirect immunofluorescence. A summary of the procedure is given in Fig. 1.

Following the establishment of these hybrid cell lines the antibody product could be subjected to further biochemical analysis and purification, 'large scale' production of antibodies carried out by injecting the cells into histocompatible mice (where they grow in ascites form and where the ascitic fluid may contain up to 1 mg/ml of specific antibody when harvested) or the hybrid cell lines frozen until further use.

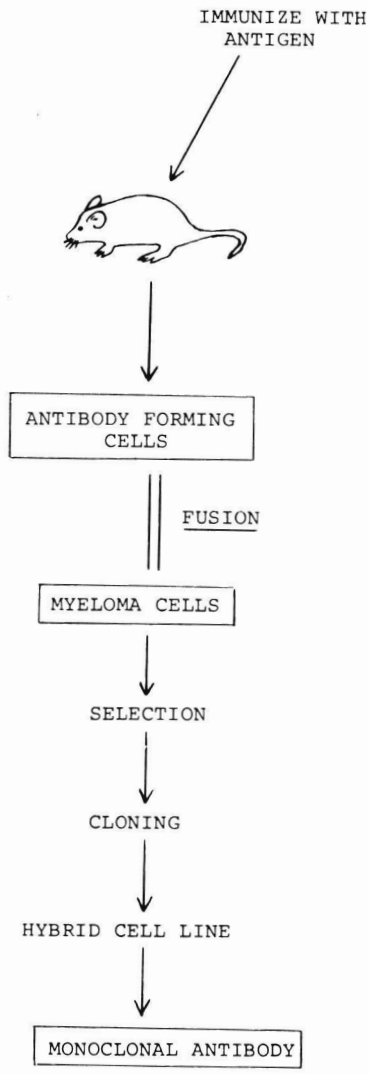

Fig. 1. Production of hybridomas

IMPORTANT FACTORS IN HYBRIDOMA PRODUCTION

Several factors need to be taken into consideration for the successful production of hybridomas:

1. **Cell properties**

a. *ontogenetic* restriction - in order to 'rescue' B cell function (ie. antibody production) it appears to be necessary to carry out the fusion with a B cell tumour (ie. myeloma cells). Conversely, preservation of T cell function in the hybrid requires that the T cells be fused with a T cell tumour (Goldsby *et al.*, 1977).

b. *phylogenetic* restriction-fusion between the same or between closely related species are preferred e.g. fusion between mouse myeloma cells and mouse or rat spleen cells produces many more successful, specific hybrids than fusions between mouse myeloma cells and human or frog cells (Kohler and Schulman, 1978).

c. *stage of cell cycle* - large, dividing lymphocytes (B cells) from the spleen are preferred to 'resting' cells for fusion with myeloma cells.

2. **Immunization protocol**

The type of antigen preparation, dose, frequency of administration and time elapsed between immunization and collection of spleen cells are important factors which subsequently influences the success of fusion procedures.

3. **Fusion conditions**

Optimal conditions for fusion (e.g. concentration of polyethylene glycol) need to be carefully determined.

4. **Screening methods**

Rapid and reliable methods to detect the antibody product of the hybrids are necessary.

RELEVANCE OF HYBRIDOMAS TO MEDICAL DIAGNOSIS AND PRACTICE

There are many aspects of medical diagnosis and practice in which hybridomas are of immediate relevance. The chief attraction lies in the resolving power of the hybridoma product: its *monoclonal* nature, its *homogeneity,* its *high affinity* and *absolute specificity.* These features together with the technique's simplicity and thus economical production costs for such antibodies will eventually benefit everyone from the general practitioner doing simple laboratory diagnostic procedures, the transplant surgeon interested in tissue typing and matching, and the research scientist involved, for example, in identification of parasite antigens. More specifically, the areas in which the hybridoma technology has been used or will be of specific value include the following:

1. **Parasitic diseases**

In parts of the world where parasitic diseases are endemic, the detection of antibodies in patients is of little diagnostic value. In relation to this problem, monoclonal antibodies would be invaluable for the detection of parasite *antigens* in infected hosts. Additionally they represent ideal reagents for identification of parasite *variants* e.g. in trypanosomes (Vickerman, 1978), *purification* of antigens from parasite extracts and identification of antigenic determinants on parasite membranes which are involved in the development of immunity to the parasite. These analyses may in turn lead to better *vaccines* against various parasites. Studies using hybridomas have already been initiated with malaria, trypanosomiasis and schistosomiasis (W.H.O., 1979).

2. **Transplantation**

Tissue typing (HLA typing) is still a cumbersome process and monospecific anti-HLA antisera are scarce and difficult to produce. To this purpose, mice could be injected with human lymphocytes or purified human HLA antigens and monoclonal anti-HLA antibodies produced by the hybridoma method as has been done with rodents (Galfre *et al.,* 1977). Truly identical antibodies produced in this way would enable a finer distinction between tissue antigens thus resulting in better matching of tissues prior to transplantation (see New Scientist, 1977; Staehelin, 1978). Additionally, these monoclonal antibodies would be of immense value to researchers attempting to unravel the nature of the association between histocompatibility antigens and human disease e.g. HLA-B27 and ankylosing spondylitis (Staehelin, 1978).

3. **Endocrinology**

Research in several institutes and (significantly) the laboratories of Hoffmann-La Roche in Switzerland has also led to the production, by the hybridoma technique, of high affinity, highly specific antibodies to HCG (human chorionic gonadotrophin), HLH (human luteinizing hormone) and HFSH (human follicle-stimulating hormone) (see New Scientist, 1977; W.H.O., 1979). The commercial availability of these reagents may result in cheaper, more reliable pregnancy testing and may also aid in the diagnosis of choriocarcinoma.

4. **Immunogenetics and Virology**

Hybridoma antibodies have been used to actually map the location of the immunoglobulin heavy chain gene in the mouse (W.H.O., 1979). It has also been used to map the surface of the influenza virus haemagglutinin with obvious implications for serotyping of the various influenza viruses (Cancro *et al.,* 1978).

Looking into the future, the application of

the hybridoma technique appear to be limitless. It could, for example be used to provide better identification of *tumour-specific* antigens and those tissue antigens associated with *autoimmune* diseases. Such reagents may also prove as most important tools for the development of *immunoprophylaxis* against a variety of human diseases.

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