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Applications of Monoclonal Antibodies in Animal Health and Production

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2.1 INTRODUCTION

The animal production industry is a profit-directed business. The goal of the animal producer is to raise healthy 'finished' animals of correct weight for market in the most cost-effective manner and the shortest possible time. The cost of producing the animal include initially obtaining it, feeding it to achieve maximum feed conversion and average daily weight gain, keeping it healthy and protecting it from disease. Obviously the health of the animal will significantly affect its productivity. The producers must also ensure that their breeding stock produces healthy offspring as often as nature permits, intervening when possible to increase production beyond natural limits. Any biotechnology aid that can help them to improve animal health, feed conversion or shorten the time to the animal achieving market weight is worth their consideration. The costs of such aids must however be weighed against the improvement in production that they provide. Biotechnology aids have been applied in many areas, including: mechanisms to increase successful pregnancies (hormones, artificial insemination, oestrus/pregnancy detection tests); disease monitoring with vaccination of pregnant animals to protect their offspring from disease; disease monitoring with vaccination of neonatal offspring and prophylactic antibiotic supplements in feed to continue this protection; and growth promoters to maximize feed conversion ratios.

Biotechnology in the form of murine hybridoma monoclonal antibodies (MAbs) has been used in numerous research applications which will ultimately benefit the animal producer in most of the above areas, and many of these have been reviewed by other authors (Booman, 1985; Nielsen *et al.*, 1986; Snyder, 1986; McCullough *et al.*, 1987; Van Brunt, 1987). Practical applications that directly affect the industry, however, include the following.

(i) MAb-based immunoassays for monitoring ovulation and detecting pregnancy in cattle (Booman *et al.*, 1984); several kits are now commercially available, including 'Calfcheck', 'Heifercheck' and 'Calfcheck Confirm' from American Diagnostic Sales, Westport, CT (Van Brunt, 1987).

(ii) MAb-based rapid immunodiagnostic assays for early identification of disease outbreaks, to enable isolation of infected animals and reduce spread of disease.

(iii) MAbs against specific infectious agents used either directly for passive immunization or indirectly (for immunopurification of 'protective' antigens) for active immunization against the infectious disease, in a range of animal species.

(iv) MAbs used as therapeutic modifiers of specific physiological functions to increase animal production, *e.g.* increasing frequency of ovulation or as growth promotors.

(v) MAbs against immunoglobulins and specific cell surface markers have potential applications in immunodiagnosis and for immunotherapy in several disease states and certain types of tumour.

Some research applications of MAbs will be discussed in this chapter, but the focus will be on those applications of MAbs that can directly benefit the animal health and production industries.

2.2 PRODUCTION AND PURIFICATION OF MONOCLONAL ANTIBODIES

Methods for production and culture of MAb-secreting hybridomas, screening of culture supernatants for the presence of specific MAbs and purification of selected MAbs from culture supernatant or ascitic fluid have been reviewed in detail elsewhere and will not be repeated here (Nielsen *et al.*, 1986; Fuller *et al.*, 1987). Two important cautionary points will however be mentioned, and will be emphasized at appropriate places later in this chapter.

(i) Before attempting to produce MAbs, the researcher should be absolutely sure of their intended application. Assay systems that will determine the suitability of MAbs for this intended application should be developed and optimized, so they can be used for testing culture supernatants as early as possible during the hybridoma production process. Many researchers screen hybridoma culture supernatants using microtitre ELISA systems. Though microtitre ELISA is a simple and convenient screening method, it will detect only MAbs that bind to target antigen coated on to a microtitre plate. Owing to their unique specificity and properties, the MAbs detected in this way may not be suitable for use in other applications. They may not, for example, react with native antigen in immunohistopathology (immunocytochemistry), with antigen in solution in sandwich (capture) ELISAs, or irreversibly bind antigen when acting as an immunoadsorbent. An example of this phenomenon was described by Thiriart *et al.* (1986), who had difficulty in obtaining neutralizing MAbs against bovine rotavirus when using an ELISA system for screening hybridoma supernatants. His group therefore developed a rapid sensitive rotavirus neutralization assay and adopted it for direct screening of primary hybridoma supernatants. Using this assay, they obtained more than 70 neutralizing MAbs from one fusion, which gave negative results when tested by their original ELISA for detecting rotavirus antibodies.

(ii) The method for production and the technique and required level of purification of the MAb will depend on its intended application. Most researchers produce MAb by ascitic culture in mice. *In vitro* production methods are, however, rapidly becoming more cost efficient. In addition, purification of MAb from ascitic fluid (which contains high levels of contaminating host mouse immunoglobulins) can often be more complex (and therefore more costly) than purification from culture supernatant that contains low concentrations of, or no, serum. The required degree of purity must be determined on the basis of the intended application; for example, high purity MAb is necessary for immunotherapy by injection, but ascitic fluid containing specific MAb may be suitable for immunotherapy by oral administration (see Section 2.5.2). The temptation to 'overpurify' MAbs should be avoided, to minimize unnecessary costs, and the 'specific activity' (selected MAb activity per mg protein) of the preparation should be monitored to ensure that it increases after each step of the purification scheme.

2.3 AREAS OF APPLICATION FOR MONOCLONAL ANTIBODIES

The applications of monoclonal antibodies (MAbs) in animal health and production may conveniently be divided into four areas: (1) immunocharacterization; (2) immunopurification; (3)

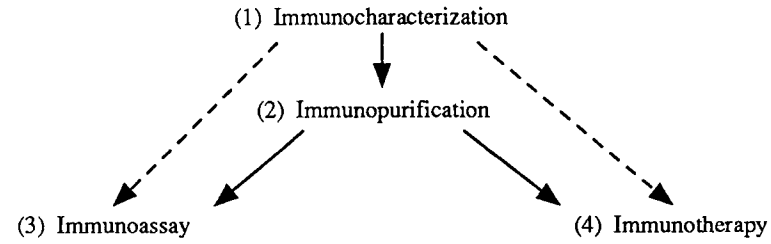

Figure 1 Interrelationship between the four main areas of application of monoclonal antibodies to animal health and production. Applications indicated by broken line arrows utilize monoclonal antibody itself; applications indicated by solid line arrows utilize immunoaffinity purified antigen

immunoassay; and (4) immunotherapy. These four areas are not however exclusive of one another, and are in fact mutually interconnected in the way shown in Figure 1.

Immunocharacterization involves characterization of the MAb and its specificity. Characterization of the MAb might involve ascertaining its isotype, affinity constant and any biological activity it may have in the presence of its target antigen; for instance neutralization (in the case of a virus), complement fixation or agglutination.

Characterization of the MAb's specificity can not only yield valuable information on the structure of its target antigen (by using, for example, Western blots or polyacrylamide gel electrophoresis of immune precipitates), but, by comparison with the MAb's biological properties, can also identify the functions of the antigen (*e.g.* neutralization, haemagglutination) and which epitopes of the antigen are involved in these functions (epitope mapping). Elucidation of the true nature of epitopes (Sela, 1969) has been aided by the use of MAbs (Van Regenmortel, 1984). Complete immunocharacterization should be performed before applying the MAb to its intended purpose.

Once characterization of the MAb and its specificity has been performed, the next area for consideration will depend on whether the desired application involves the MAb itself or antigen that has been immunoaffinity purified using the MAb (Figure 1). This will be true regardless of whether the intended use is in immunoassay or immunotherapy. If the MAb itself is to be used, then the application indicated by the broken line arrow in Figure 1 would be pursued immediately. If the antigen against which the MAb is directed is to be utilized, then an immunopurification step is required. This necessitates immobilization of the MAb on a suitable solid phase, normally a gel matrix. It is not the intention of this chapter to advise on the choice of solid phase matrix, as their suitability may vary, depending on the MAb and the target antigen to be purified. CNBr-activated agarose may be suitable for some systems whilst others may require chemically substituted gels or those with spacer arms. It should be stressed that not all MAbs are suitable for use as solid phase immunoadsorbents. Each MAb must be evaluated for its suitability in its intended use as early as possible during its production.

The choice of eluent for affinity purification of antigenic components using MAb immunoadsorbents must be carefully considered. MAbs and their target antigens can be denatured by exposure to commonly used eluents, leading the researcher to conclude that the MAb itself is not suitable for immunopurification. Individual treatment of the crude antigen preparation and the MAb itself with the working concentration of various eluting agents (prior to application to the solid phase immunoadsorbent matrix), followed by testing each treated crude antigen and MAb to ascertain whether its immunological reactivity has been reduced, can avoid wasted time and effort.

Immunoassay falls into two areas, depending on whether the MAb itself is to be used for antigen detection, or whether an antigen, affinity purified using the MAb on a solid phase immunoadsorbent, is to be used for antibody detection. Both applications may involve several different techniques; the former includes immunohistopathology (immunocytochemistry), particle agglutination (particles coated with MAb), immunoradiometric assay, radioimmunoassay, sandwich ELISA or competitive (inhibition) ELISA; the latter application includes particle agglutination (particles coated with antigen), complement fixation, ELISA or competitive (inhibition) ELISA. Excellent descriptions of these techniques have been published elsewhere (Nielsen *et al.*, 1986; Winston *et al.*, 1987) and will not therefore be duplicated here. The previous caution will, however, be repeated. Not every MAb will be suitable for use in all of these immunoassay techniques; thus careful screening of MAbs for suitability to their intended purpose should be performed as early as possible during production. This caution is equally applicable to the areas of immunotherapy described in the next paragraph.

Immunotherapy using MAbs will be defined, for the purposes of this chapter, as comprising three areas: (i) the use of antigen (native or recombinant), affinity purified using a MAb solid phase immunoadsorbent, for active immunization against disease; (ii) the use of anti-idiotype MAbs for

active immunization against disease; and (iii) the use of MAb specific to an epitope on an infectious agent, cell surface antigen, or biologically active molecule (*e.g.* a hormone), either by itself or conjugated to an effector molecule, for passive immunization against an infectious disease or tumour, or for immunomodulation (immunoregulation) of the immune or endocrine systems.

It has been noted that all MAbs require immunocharacterization before use in any application, and that immunopurification will be necessary before an affinity purified antigen can be used. Therefore in the remainder of this chapter, rather than consider each of the four areas defined in Figure 1 individually, the applications of MAbs in animal health and production will be discussed under the two headings of immunoassay and immunotherapy, each being divided into appropriate subsections.

2.4 IMMUNOASSAY

2.4.1 Large Animal Production

2.4.1.1 Parasites

The antigenic structure of parasites seems complex when compared to the relative simplicity of many infectious agents, and this complexity presents a unique challenge to the use of MAbs in their characterization and control. Parasitic infections severely influence animal health and productivity, emphasizing the need to apply every available biotechnological tool to this field. Further, much of the knowledge gained in research into these areas of animal health may be directly applicable to control of similar diseases in humans. The applications of MAbs in parasitic diseases have been reviewed with respect to diagnosis (Gamble, 1984) and vaccine development (Gamble and Zarlenga, 1986; Gamble, 1987). However, two publications in these fields are noteworthy as they illustrate the relationships between MAb applications shown by solid line arrows in Figure 1.

Gamble and Graham (1983, 1984) produced MAbs for immunocharacterization of *Trichinella spiralis* antigens, then used a selected MAb to immunoaffinity purify a stichocyte antigen. This 'trichinella-specific' antigen was then utilized in an ELISA for testing swine sera, which completely eliminated false positive reactions (caused by the presence in sera of cross-reacting antibodies against other parasites) but identified pigs infected with trichinosis.

Wright *et al.* (1983) also used MAbs to characterize antigens of *Babesia bovis*, and employed selected MAbs to immunoaffinity purify *B. bovis* antigen. These authors went on to demonstrate that active immunization with one of these immunoaffinity purified antigens protected susceptible calves against challenge with virulent *B. bovis*.

MAbs have also been employed for antigen characterization and detection in *Trypanosoma cruzi* (Arauio *et al.*, 1982), *Fasciola hepatica* (Hanna and Trudgett, 1983) and *Theileria sergenti* (Kobayashi *et al.*, 1987).

2.4.1.2 Neonatal diarrhoea

It is in the field of neonatal diarrhoea research that MAbs have made some of their greatest contributions to animal health. MAb technology has been applied to most of the major pathogens that cause scours in neonatal calves, piglets and lambs. Greenberg *et al.* (1983) demonstrated that MAbs against the 42 kDa major structural protein of rotavirus could be used for preliminary serotyping of strains from different animal species, and Sabara *et al.* (1985) used MAbs to map the epitope on this glycoprotein that is involved in neutralization of bovine rotavirus and virus attachment to cells during infection. Sonza *et al.* (1983) and Thiriart *et al.* (1986) also produced neutralizing MAbs against rotavirus, whilst direct detection of rotavirus in porcine faecal specimens was achieved by Liprandi *et al.* (1986), who used their MAb against the 45 kDa group specific antigen in a double sandwich indirect ELISA system.

Development of a MAb sandwich (capture) ELISA system for direct detection of bovine enteric coronavirus (BEC) in faecal specimens from scouring calves was reported by Crouch *et al.* (1984). Crouch and Acres (1984) modified this ELISA system to demonstrate shedding of both coronavirus and rotavirus in faeces of clinically normal cows, either as free viral antigen or as viral antigen–bovine immunoglobulin complexes. Their data suggested that subclinical infection of cows with rotavirus and coronavirus is common, possibly providing a source for infection of the neonate. ELISA systems of this type could provide important epidemiological information on enteric virus infections, and suggest means for improving management of neonatal diarrhoea outbreaks. These researchers also

utilized their BEC-specific MAbs to show that the 120 kDa structural polypeptide of BEC was involved in both virus neutralization and haemagglutination reactions (C. F. Crouch, personal communication).

MAbs against transmissible gastroenteritis coronavirus of swine have been used for defining its antigenic structure (Laude *et al.*, 1984) and for rapid diagnosis (An *et al.*, 1986). Jenkins *et al.* (1985) also employed monoclonal antibodies as diagnostic tools for swine diarrhoea, in this case for swine dysentery caused by the spirochaete *Treponema hyodysenteriae*.

MAbs have been used extensively for studying enterotoxigenic *Escherichia coli* (ETEC) that cause neonatal diarrhoea in calves and piglets. A rapid and specific MAb competitive ELISA for detecting *E. coli* heat-stable enterotoxin in culture supernatants was developed by Thompson *et al.* (1984). Other authors utilized MAbs to study the fimbriae or pilus antigens of ETEC that are involved in adhesion of the bacterial cell to the lining of the small intestine. Foged *et al.* (1986) demonstrated different serotypes of K88 fimbriae, while Schifferli *et al.* (1987) used MAbs to probe subunit and polymer-specific epitopes on 987P fimbriae. Morris *et al.* (1985b) investigated the presence of K99 fimbriae on ETEC from calves, piglets and lambs with a MAb bacterial agglutination test, and Mills and Tietze (1984) employed another MAb in a sandwich ELISA to identify K99-positive ETEC culture isolates from calves. Direct detection of ETEC fimbrial antigens in faecal specimens would be advantageous, in avoiding the necessity for culture. MAb ELISAs for directly detecting K88 fimbrial antigen in swine faecal specimens were described by Mills *et al.* (1983); for direct detection of K99 in bovine faeces by Holley *et al.* (1984); and for direct detection and quantitation of K99 and F41 fimbrial antigens in bovine faeces by Raybould *et al.* (1987).

2.4.1.3 Respiratory diseases

In the field of virally induced respiratory diseases, two publications are noteworthy as they identify viral antigens that may be important in protection against disease. Both reports describe the use of selected MAbs for characterizing the function of certain glycoprotein antigens of bovine herpes virus type-1 (BHV-1) (infectious bovine rhinotracheitis virus). Van Drunen Littel-Van Den Hurk and Babiuk (1985) used MAbs to identify which BHV-1 glycoproteins were responsible for virus neutralization and complement dependent lysis of virus infected cells, and went on to map which epitopes on each of the two glycoproteins were involved in these functions. The authors further demonstrated that a combination of MAbs had a synergistic effect on virus neutralization, showing that more than one epitope was involved in this biological property. Chang *et al.* (1986) also used MAbs to demonstrate which BHV-1 glycoproteins are involved in virus neutralization; but unfortunately differences in nomenclature assigned by the two groups prevent direct comparison of their data.

Though MAbs have assisted in our understanding of BHV-1, they have not yet fulfilled our expectations with respiratory bacteria that infect cattle, sheep or swine. MAbs against *Pasteurella haemolytica* (the other infectious agent implicated with BHV-1 in the 'shipping fever' syndrome of calves) or its cytotoxin, *Pasteurella multocida*, *Haemophilus pleuropneumonia*, *Bordetella bronchiseptica* or respiratory Mycoplasma species have not, to date, contributed significantly to solving the problems associated with accurate diagnosis or successful therapy of these bacterial respiratory pathogens of food-producing animals.

2.4.1.4 Brucellosis

One disease that has confounded both animal producers and researchers for many years is bovine brucellosis. National schemes for eradication of bovine brucellosis have been in place for 40 years in the US, 30 years in Canada and between 15 and 20 years in various other countries. Most eradication schemes involve vaccination of cattle followed by serodiagnostic testing and slaughter of animals giving positive test results. The success of many eradication schemes has been thwarted by the inability of the serodiagnostic tests employed to discriminate between antibody titres resulting from active infection with *Brucella abortus* and residual antibody titres remaining as a result of vaccination against the disease. Countless years of research into improving serodiagnostic tests for bovine brucellosis culminated with the advent of MAb technology. The earliest publications in this area reported only the production of MAbs against *B. abortus* and its surface antigens (Holman *et al.*, 1983; Quinn *et al.*, 1984; Schurig *et al.*, 1984), though these authors did discuss the potential applications of their MAbs in serodiagnosis of bovine brucellosis. Bundle *et al.* (1984) employed

MAbs to confirm the serological cross-reactivity between the LPS O-antigen of *B. abortus* and *Yersinia enterocolitica*, type 0:9, that had first been reported by Ahvonen *et al.* (1969), and confirmed by Diaz and Jones (1973). The first report describing the use of a MAb for attempting to improve serodiagnosis of brucellosis was by Gorrell *et al.* (1984). These authors used a MAb raised against a virulent strain of *B. abortus* in an ELISA for the detection of antibody against virulent *B. abortus*. Their ELISA did not however, discriminate between sera from infected and vaccinated cattle any better than the existing serodiagnostic tests. Sutherland (1985) incorporated a pair of MAbs against *B. abortus* in a competitive ELISA, but failed to obtain improved discrimination compared to existing serodiagnostic tests. Bundesen *et al.* (1985) produced a panel of MAbs against *B. abortus* cell surface antigens which had two distinct sets of characteristics. One group of these MAbs agglutinated bacterial cells and reacted with soluble preparations of lipopolysaccharide. The second group of MAbs were not capable of agglutinating bacterial cells or binding to soluble lipopolysaccharide, but did react in an ELISA system with an antigen present in ultrasonicated brucella cells. Two of these MAbs actually exhibited differential reactivity with ultrasonicates from virulent and non-virulent strains of *B. abortus* and therefore showed potential for improved serodiagnostic tests. The same authors (Rylatt *et al.*, 1985) selected MAbs from the group described in their previous paper that agglutinated bacteria and reacted with soluble preparations of lipopolysaccharide, to prepare horseradish peroxidase conjugates for use in a competitive ELISA system for screening bovine sera. This competitive ELISA system did improve discrimination between sera from infected and vaccinated cattle compared to existing serodiagnostic tests. Sutherland and Hollander (1986), similarly compared the ability of a competitive ELISA (described in Sutherland's 1985 paper) with an existing serodiagnostic test, the complement fixation test, and concluded that their competitive ELISA also enabled improved discrimination between infected and vaccinated animals. Another peroxidase-labelled MAb ELISA was recently described by Henning *et al.* (1987). It is hopeful that by the cumulative efforts of these different groups, the use of MAbs will enable achievement of the goal for absolute discrimination between animals infected with brucellosis and those vaccinated against the disease.

2.4.1.5 Other infectious diseases

Many groups have produced MAbs against infectious agents and used them either for immunocharacterization or immunodiagnosis. Significant advances in our knowledge of the structure, epidemiology and detection of several important viruses have been achieved by the use of MAbs. MAbs against rabies virus have been employed for molecular analysis of natural antigenic variants (Flamand *et al.*, 1980; Koprowski and Wiktor, 1980; Rupprecht *et al.*, 1987), to distinguish between street, vaccine and laboratory strains (Smith *et al.*, 1984) and for antigenic characterization of street virus strains (Tollis *et al.*, 1987). MAbs against foot-and-mouth disease virus have enabled antigenic characterization and epitope mapping (McCullough and Butcher, 1982; Robertson *et al.*, 1984). Production of MAbs against the major core protein of Maedi-Visna virus has enabled development of a one-step competitive ELISA for detecting serum antibodies to ovine and caprine lentiviruses (Houwers and Schaake, 1987), and characterization of Scrapie prion proteins has also been attempted using MAbs (Barr and Prusiner, 1986). The use of MAbs against bovine viral diarrhoea virus in immunofluorescent antibody techniques has aided preliminary serological characterization of strains (Peters *et al.*, 1986), and both indirect and competitive ELISA systems utilizing MAbs detect antibodies against the virus in bovine sera (Juntti *et al.* 1987). MAbs against swine fever virus have enabled development of ELISA and immunocytochemistry systems for routine differential diagnosis between swine fever virus infection or vaccination and bovine viral diarrhoea infection (Wensvoort *et al.*, 1986). MAbs against bluetongue virus have been used for analysis of serotype restricted and unrestricted viral antigenic determinants (Appleton and Letchworth, 1983), for detection of virus group-specific antibodies in cattle and sheep by a MAb-blocking (competitive) ELISA (Anderson, 1984, 1985) and for demonstration of viral antigen in infected tissues (Cherrington *et al.*, 1985).

Bovine leukemia (leukosis) virus (BLV) infection of cattle is another area where MAb-based ELISA systems have enabled detection of virus-specific serum antibody. (Portetelle *et al.*, 1983; Mammerickx *et al.*, 1984; Portetelle *et al.*, 1984a, 1984b). These authors utilized MAb against glycoprotein 51 (gP51) whilst Logan *et al.*, (1986) developed MAbs against gP60 (which is probably the same envelope glycoprotein as gP51) and p24 in their studies. Yoneda *et al.* (1986) attempted BLV antigen detection by using a BLV-specific MAb in a colorimetric cytotoxicity assay to detect tumour-associated antigens on BLV infected cells in peripheral blood samples from leukemic cattle, while

Hamada *et al.* (1984) and Djilali and Parodi (1986) demonstrated BLV-transformed sheep cells with their BLV-specific MAbs. MAbs have also been used for studying tumour-associated antigens on bovine lymphosarcoma cells by Aida *et al.* (1987).

MAbs have contributed to our understanding of certain non-viral infectious agents of large animal species, including *Mycoplasma hyorhinis* infection of swine (Wise *et al.*, 1984), *Chlamydia psittasi* infection of sheep (Delong and Magee, 1986), and detection of *Streptococcus agalactiae* (Ainsworth and Capley, 1986) and *Mycobacterium bovis* (Morris *et al.*, 1985a; Boothby *et al.*, 1986) in cattle.

2.4.1.6 Components of the immune system

MAbs have been produced against a variety of mediators of animal immune responses, including immunoglobulins and immune cell surface markers. MAbs against immunoglobulins have been used for three main applications: (i) characterization and mapping of epitopes involved with various immunoglobulin functions; (ii) quantitating levels of different immunoglobulin classes and subclasses as a guide to the immune status of the animal; (iii) quantitating differential responses in immunoglobulin classes or subclasses against specific infectious agents.

The use of MAbs in the first application, for mapping sheep IgG_1 and IgG_2 subclasses was described by Beh (1987) and the first two applications were alluded to by various groups who produced MAbs against bovine IgG_1 (Fleenor *et al.*, 1984), bovine IgG_2 (Srikumaran *et al.*, 1982) and porcine IgM (Paul *et al.*, 1985). The third application has been described using isotype-specific MAb ELISA systems for detecting bovine immunoglobulins against rotavirus (Van Zaane and Ijzerman, 1984) and porcine virus-specific immunoglobulins (Van Zaane and Hulst, 1987).

MAbs against cell surface markers have also been used in a number of areas. Pinder *et al.* (1980a, 1980b) demonstrated the presence of bovine IgM in serum and on cell surfaces with a MAb specific to this immunoglobulin. Production of MAbs against major histocompatibility complex products for bovine tissue typing purposes was attempted by Spooner and Pinder (1983) with little success, and by Letteson *et al.* (1983) who did succeed in producing a xenogeneic monoclonal antibody against a bovine 'Ia-like' antigen. The ability to distinguish between T and B lymphocytes using MAbs would have potential in either studying cellular immune responses or determining the immune status of animals, and was investigated with cattle by Lewin *et al.* (1984a, 1984b, 1985) and with swine by Lunney (1984) and Lunney *et al.* (1984). Further differentiation of T lymphocytes into subpopulations by the use of MAbs was reported with bovine cells by Rabinowsky and Yang (1984) and with porcine cells by Jonjic and Koszinowski (1984). Potential immunotherapeutic applications of all these MAbs will be discussed in Section 2.5.

2.4.1.7 Toxins and drug residues

MAbs have been used in developing immunoassays for the detection of toxins in animal feed, and for detection of residual drugs in animal food products. MAb ELISA systems have been described for detecting various mycotoxins, including aflatoxin B-1 (Candlish *et al.*, 1985), T-2 toxin and ochratoxin A (Kawamura *et al.*, 1986), zearalenone and α-zearalenol (Dixon *et al.*, 1987). It has previously been mentioned that anabolic drugs may be employed as growth promoters in animals, and animal feed may be supplemented with antibiotics for prophylactic control of infectious disease. Carter *et al.* (1984) prepared MAbs against the anabolic agent zeranol, and suggested applications of this antibody in immunoassays for monitoring residues of zeranol in animal products. Several authors have developed MAbs against antibiotics and suggested their use in immunoassays for detecting the presence of residual antibiotic in animal products, including Van de Water *et al.* (1987), who used a MAb against chloramphenicol for development of a competitive ELISA for determination of chloramphenicol residues in swine muscle tissue.

2.4.2 Poultry Production

There were few publications on applications of MAbs to poultry diseases until the early 1980s, in spite of the fact that studies of the chicken immune system played a key role in our current understanding of immune responses. MAbs against species of avian coccidia were first produced in 1982 (Danforth and Augustine, 1982), but their use for immunodiagnostic purposes has only been reported more recently. MAbs against *Eimeria tenella* microgametocytes have been employed for

immunofluorescent antibody technique (Laxer *et al.*, 1987), and MAbs against surface antigens of *Eimeria* sporozoites in both immunofluorescent antibody tests and immunoelectron microscopy (Augustine and Danforth, 1987). Other authors have applied MAbs to differentiating strains of avian leukosis virus in chickens (Lee *et al.*, 1986); for differentiating antigenic variant strains of avian bronchitis virus by ELISA (Koch *et al.*, 1986); for characterizing antigens and serotyping isolates of Marek's disease virus and herpes virus of turkeys (Ikuta *et al.*, 1983; Lee *et al.*, 1983; Hirose *et al.*, 1986); and for rapid diagnosis of infectious bursal disease by avidin-peroxidase staining of cell smears from chicken embryo fibroblast cultures and Bursa of Fabricius (Cho *et al.*, 1987). Russell and Alexander (1983) studied antigenic variation of Newcastle disease virus strains with MAbs, whereas Srinivasappa *et al.* (1986) used a MAb specific to vaccine strains of Newcastle disease virus in an indirect ELISA for differentiating between vaccine and virulent field strains. Van Den Hurk (1986) produced MAbs against haemorrhagic enteritis virus (HEV) of turkeys and incorporated these antibodies into an ELISA for quantitating HEV antigens in tissue extracts. This assay was more sensitive than the commonly employed agar gel precipitation test and was therefore used to quantitate HEV antigen in experimentally infected turkeys.

2.4.3 Fish Farming

Our knowledge of the immune system of fish and fish diseases is extremely limited when compared to our knowledge of large animals. Nevertheless, fish farming (aquaculture) is becoming an increasingly important food production industry, and may play a significant role as a food source in the future. For this reason, application of the latest biotechnological advances, including MAbs, to the aquaculture industry, is extremely important. This is, in fact, occurring, and in addition to the application to the industry of areas of biotechnology beyond the scope of this chapter, MAbs are being adopted for purposes of immunoassay and possibly immunotherapy (see Section 2.5). MAb-based ELISAs have been used for studies of *Vibrio anguillarum* strains (Goerlich, 1987) and for rapid diagnosis of clinical cases of enteric redmouth (*Yersinia ruckeri*) and furunculosis (*Aeromonas salmonicida*) (Austin *et al.*, 1986) in fish farms. MAbs have also been employed for analysis of lymphocyte receptors (Fiebig *et al.*, 1983) and characterization of lymphocyte populations (Egberts *et al.*, 1983) in carp, for immunopurification of salmon prolactin, and for development of sandwich ELISA systems for both salmon prolactin and somatotropin (Furuya *et al.*, 1987).

2.5 IMMUNOTHERAPY

2.5.1 Active Immunization With Anti-idiotype Monoclonal Antibodies

An anti-idiotype antibody (anti 'anti E' antibody in Figure 2) has, as shown diagrammatically in Figure 2, specificity to the antigen-combining amino acid epitope sequence (of the folded molecule) of the hypervariable region of the antibody ('anti E' antibody in Figure 2) raised against the original antigen epitope (E in Figure 2). The principle of anti-idiotype antibodies and their use for inducing protective immunity may be simplified in the following way, with reference to Figure 2. An animal (A in Figure 2) hyperimmunized with antigen X will produce antibodies with a wide range of specificities for epitopes on X, but a very small number of these antibodies will be specific to epitope E ('anti E' antibody in Figure 2). The epitope-combining site of these antibodies will contain the 'mirror image' of epitope E. If these 'anti E' antibodies are isolated either by immunoaffinity purification (on an epitope E immunoadsorbent) or by cloning the cell line secreting them, and the pure (or monoclonal) 'anti E' antibody is then used to hyperimmunize a mouse (B in Figure 2), then amongst the countless antibody specificities produced against epitopes on this 'anti E' antibody molecule, a very small number will be specific to the epitope that was the 'mirror image' of the original epitope E (anti 'anti E' antibody in Figure 2). As the epitope-combining site of these anti 'anti E' antibodies is the 'mirror image' of the 'mirror image' of epitope E, then it should be antigenically identical to the original epitope E. This anti 'anti E' antibody is an anti-idiotype antibody and can be isolated by hybridoma cell fusion and cloning procedures. If this pure monoclonal anti-idiotype antibody is then injected as an active immunogen into another species of animal (C in Figure 2), the animal should produce a proportion of antibodies that will react with the original epitope E (D in Figure 2). If E was an epitope on a virus, involved in virus neutralization, then this antibody may neutralize the virus (D in Figure 2).

Anti-idiotype MAbs have been evaluated as immunogens for active immunization by several

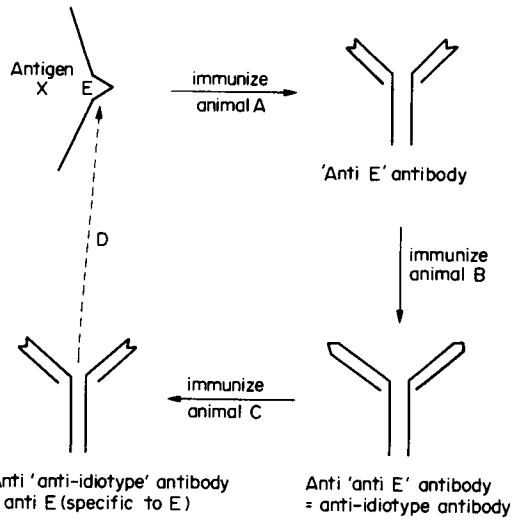

Figure 2 Principle of anti-idiotype antibody generation and method of use for inducing specific immunity against a particular epitope

groups (*e.g.* Gurish *et al.*, 1986; Kennedy *et al.*, 1986; Hiernaux, 1988) and have several advantages. The anti-idiotype MAb carries no risk of contamination with nucleic acid of the virus against which protection is desired, and has low toxicity. Anti-idiotype MAbs can mimic carbohydrate or lipid antigenic determinants (epitopes) that cannot easily be produced by recombinant DNA technology and which, in their native form, may be poorly immunogenic. Further, anti-idiotype MAbs express fewer antigenic determinants than, for instance, bacterins, thus reducing the chance of autoimmune responses, and they provide a continuous source of a standard immunogen. Anti-idiotype MAbs also have disadvantages. Like a dead vaccine, they are rapidly cleared from the injection site in the body, and their effectiveness at inducing cellular immunity in different species has not been established. An anti-idiotype MAb may not exactly mimic the original epitope, and even if it does, this single epitope may not induce protective immunity. Finally, in generating anti-idiotype MAbs, very few useful antibody types are produced at each step, and extensive testing and absorption of preparations at each step may be necessary to ensure removal of any antispecies or antiallotype activity.

The use of anti-idiotype MAbs for inducing protective immunity against infectious agents has been investigated by several groups. Reagan *et al.* (1983) produced anti-idiotype MAbs against a neutralizing MAb specific to glycoprotein G of rabies virus. Immunization of mice with these anti-idiotype MAbs resulted in a rabies-neutralizing antibody response. Stein and Sonderstrom (1984) achieved protection against *E. coli* K13 infection in mice by neonatal administration of either specific anti-idiotype or idiotype antibody. In the field of animal production,various antibovine idiotype MAbs that mimic epitopes on bovine *Streptococcus* and *Staphylococcus* strains have been described by Arulanandam *et al.* (1985) and Arulanandam and Goldsby (1987), and induction of both antibody and cellular immune responses against *Eimeria tenella* has been demonstrated in chickens by administration of anti-idiotype MAbs (Bhogal *et al.*, 1987).

2.5.2 Passive Protection against Infectious Diseases

Successful passive protection of animals against a virus-induced disease was first reported by Letchworth and Appleton (1983) who used a MAb against bluetongue virus to protect sheep and mice from intravenous challenge with the homologous virus serotype. This MAb, an IgG_{2a}, was shown *in vitro* to neutralize virus, to inhibit virus haemagglutination and to precipitate bluetongue virus polypeptides 2 and 3. In 1984, Jeggo *et al.* also described partial protection of sheep against bluetongue challenge by administration of MAb against the homologous virus serotype. The ability of MAbs against pseudorabies glycoproteins to passively protect pigs from intranasal challenge with pseudorabies virus was independently demonstrated by Marchioli *et al.* (1986) and Sadowski *et al.* (1986b).

The first report of successful passive protection of calves against challenge with enterotoxigenic *Escherichia coli* (ETEC) by MAb was made in 1983, by Sherman *et al.* These authors demonstrated

that oral administration of ascitic fluid containing MAb specific to the K99 fimbriae (pilus) antigens (which are involved in adhesion of the ETEC cell to the gut wall during colonization) of ETEC, prevented death and reduced clinical symptoms resulting from oral challenge with K99-positive ETEC. Sadowski *et al.* (1986a) demonstrated a similar protective ability of a 987P fimbriae (pilus)-specific MAb to reduce mortality due to 987P-positive ETEC induced colibacillosis in neonatal pigs, and Foged *et al.*, (1986) suggested that their K88 fimbriae (pilus)-specific MAb might also protect against K88 positive ETEC, but no supportive experimental data were included in their paper.

The possibility of using MAbs for passive protection of neonatal calves against another important diarrhoea-causing agent was investigated by Thiriart *et al.* (1987). These authors attempted to protect neonatal calves against experimental infection with bovine rotavirus by oral administration of rotavirus-specific MAbs. Unfortunately, though their MAbs individually neutralized homologous bovine rotavirus *in vitro*, they did not protect *in vivo*, even when combinations of different MAbs were used. The failure of these MAbs to provide passive protection against rotavirus challenge is likely due to the difference between the pathogenesis of ETEC and rotavirus-induced diarrhoea. Fimbriae (pilus)-specific MAbs probably protect by binding to fimbriae, on ETEC cells, that are involved in adhesion of the ETEC to the epithelial cells of the small intestine, thus preventing adhesion and subsequent colonization. Rotavirus, however, infects intestinal villous epithelial cells, and once cell entry has occurred, the virus may no longer be accessible to neutralizing MAb.

2.5.3 Increased Production and Growth Promotion

MAbs have been produced against a variety of hormones, including bovine progesterone (Booman *et al.*, 1984), bovine and porcine insulin (Schönherr *et al.*, 1984), pregnant mare serum gonadotropin (PMSG) (Dieleman *et al.*, 1987), bovine somatotropin (Krivi and Rowold, 1984) and bovine somatostatin (Buchan *et al.*, 1985). In view of the successes in the use of MAbs for 'neutralizing' infectious agents *in vivo*, provided they are accessible to the MAbs in the body, it is not unreasonable to suggest that these hormone-specific MAbs could have potential for *in vivo* therapeutic immunomodulation of various physiological functions. In fact, PMSG-specific MAb was successfully used by Dieleman *et al.* (1987) for modifying the number of ovulations occurring in cows.

Growth promotion in animals has been achieved for many years using various anabolic agents (*e.g.* Ralgro). More recently, there has been considerable interest in the use of growth hormones or 'somatotropins' as growth promoters (Figure 3). Rather than extracting these hormones from pituitary glands, the genes for porcine somatotropin (pST) and bovine somatotropin (bST) have been cloned and expressed in bacteria, and the genetically engineered hormones are now produced in large quantities using fermenter culture (Summers, 1988). Several companies have made major investments in this technology (Ingenthron, 1988; Simkins and Eggert, 1988; Summers, 1988) and extensive studies of the effect of recombinant pST on growth rates in pigs and of recombinant bST on milk production in dairy cattle are currently underway by Pitman-Moore and American Cyanamid respectively (Summers, 1988; Simkins and Eggert, 1988). The use of somatotropins for increasing animal production does have disadvantages, however, due to the putative species-specificity of their effects (Summers, 1988) and the possibility of residual recombinant material in meat and dairy products (Lister, 1983).

An alternative approach to growth promotion is the use of antibodies against somatostatin (Figure 3). Neutralization of somatostatin should result in a net increase in levels of native somatotropin produced by the pituitary gland. Active immunization of sheep against somatostatin conjugates has been shown effective in producing significant increases in weight gain and improvements in food utilization efficiency (Spencer, 1986), whereas active immunization of lactating goats significantly increases their milk production (Spencer, 1986; Garssen *et al.*, 1987). Active immunization against somatostatin has several problems however, including the low immunogenicity of somatostatin, due to it being a naturally occurring hormone recognized as 'self' by the immune system, and the variability of somatostatin-specific immune responses between individual animals and in different animal species (Spencer, 1986).

Passive immunization against somatostatin was suggested as an alternative approach to growth promotion by Spencer (1986), and was shown to be effective using somatostatin-specific goat antiserum by Buonomo *et al.* (1987). Polyclonal antibody against somatostatin was also used by Plisetskaya *et al.* (1986) to investigate its effect *in vivo* on insulin and glucagon levels in coho salmon. Somatostatin-specific MAbs have been produced, and their binding to somatostatin demonstrated *in vitro* (Buchan *et al.*, 1985) and *in vivo* (Seal *et al.*, 1987). Though no publications on the efficacy of these MAbs as growth promotors have appeared in the scientific literature, other reports indicate that

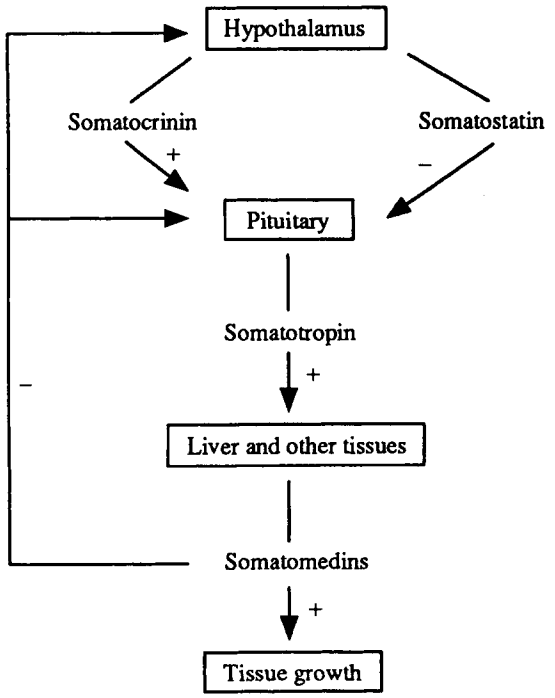

Figure 3 Regulation of animal growth by hypothalamus and pituitary hormones: + indicates stimulatory effect and − indicates inhibitory effect

they may be equally as effective as recombinant somatotropins, but without their disadvantages (Maccecchini, 1986; Marbery, 1988).

2.5.4 Immunomodulation and Tumour Therapy

MAbs have been produced against a range of surface markers on immune cells in animals (Pinder *et al.*, 1980a, 1980b; Letteson *et al.*, 1983; Spooner and Pinder, 1983; Jonjic and Koszinowski, 1984; Lewin *et al.*, 1984a, 1984b; Lunney, 1984; Lunney *et al.*, 1984; Rabinowsky and Yang, 1984; Lewin *et al.*, 1985). These MAbs, together with others of suitable specificity, might be usable *in vivo* for reducing the numbers of selected types of immune cells (selective depletion), to achieve immunomodulation in a variety of immunological disorders or disease states. Certain groups have, in fact, investigated the potential applications of MAbs in some of these areas (Pavlov *et al.*, 1982; Steplowski *et al.*, 1983; Greene *et al.*, 1985; Steele *et al.*, 1988). Selective depletion of cell populations using suitable MAbs might have application in various lymphoproliferative diseases in animals. In the field of human medicine, MAbs against tumour cell surface markers are used to aid diagnosis and monitoring of disease progression, in addition to immunotherapy. Excellent reviews have been published on the applications of MAbs in the management of human tumours (Abrams and Oldham, 1985; Woolfenden and Larson, 1985; Houghton and Scheinberg, 1986; Schlom, 1986) and many of these uses may be applicable in veterinary medicine. The cost effectiveness of MAb therapy in immunomodulation and tumour management will determine the extent of its use in improving health and production of food-producing animals.

2.5.5 Non-murine Monoclonal Antibodies

One of the problems associated with the use of murine MAbs for immunotherapy in non-murine animal species is development of immunity, by the treated animal, against the murine MAb. In the case of repeated injections or prolonged MAb therapy, it is likely that antibody, produced by the treated animal against the murine MAb, neutralizes it before the MAb can exert its desired effect. Further, if the MAb is being used for therapeutic purposes where cooperation between antibody and cells, complement, or other accessory immunologic defence mechanisms are important, the therapy

may be more effective if MAb of the same species as the animal under treatment is used. One method of overcoming the first problem is the use of tolerogenic poly(ethylene glycol) derivatives of the xenogeneic MAb (Wilkinson *et al.*, 1987). Another approach, which addresses both problems, is the use of MAbs of the same species as the animal under treatment.

Production of bovine MAbs by bovine–murine hybridomas was first reported by Srikumaran *et al.* (1983, 1984). Unfortunately, these bovine monoclonal immunoglobulins were of unknown specificity. Tucker *et al.* (1984) re-fused bovine–murine hybridomas to obtain lines secreting bovine MAb that the authors concluded 'was probably directed' against Forssman antigen on sheep erythrocytes. Data confirming this specificity were not however presented. Raybould *et al.* (1985a) published the first report describing production of bovine MAbs with a defined specificity against an infectious agent. These authors produced a bovine–murine hybridoma that secreted bovine IgG_2 specific to a 26 kDa structural polypeptide of bovine enteric coronavirus. In the same year, Goldsby *et al.* (1985) described bovine–murine hybridomas secreting bovine MAbs against *Staphylococcus aureus, Escherichia coli*, and *Streptococcus agalactiae*, and Raybould *et al.* (1985b) reported production of a porcine–murine hybridoma that secreted porcine monoclonal IgG against a 50 kDa polypeptide of enterotoxigenic *Escherichia coli*. Sheep–mouse hybridomas were originally produced in 1981 (Tucker *et al.*, 1981), but these hybridomas failed to secrete MAb. In 1987, however, Groves *et al.* reported successful production of stable sheep–mouse hybridomas that secreted sheep MAb against testosterone. Production of a bovine MAb with potential for immunotherapeutic use was achieved by Anderson *et al.* (1987), who re-fused bovine–murine hybridomas to obtain stable lines that secreted bovine MAbs against F5 K99 fimbriae (pili) of ETEC. Comparison of the efficacy of these bovine MAbs with the K99-specific murine MAb described by Sherman *et al.* (1983), for passive protection of calves against ETEC challenge, would be a valuable experiment.

2.6 CONCLUSIONS

The technology for MAb production has been in existence for more than 12 years, yet their application to animal health and production is clearly still in its infancy. Advances in human medicine usually result from new technology developed in animal models. In the use of MAbs in animal health, the reverse may be true; particularly in therapeutic applications, human medical research may provide the clues for future MAb applications in animal health. Recent advances in hybridoma technology, such as the ability to generate other species of MAbs, and improvements in *in vitro* production methods, will expand their use even further.

This chapter has considered many of the uses of MAbs in improving health and productivity of food-producing animals. It has shown their versatility and suggested their future value. The chapter began by stressing the business aspect of the animal health and production industries and it will end with the same sentiment; the true value of MAb applications in animal health and production will be realized when the technology for their production and various uses reaches a point where, to the producer, they are truly cost effective methods of improving profits.

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