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## **Application of Monoclonal Antibodies in Animal Production: A Review**

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### **ABSTRACT**

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The hybridoma technology for production of monoclonal antibodies circumvents many of the constraints associated with the use of conventional antisera, and consequently broadens the areas of application of antibodies in animal sciences. In the present review, the potential usefulness of monoclonal antibodies in animal production – with emphasis on reproduction – is discussed, including the inherent limitations of the current technology and the improvements that can be foreseen within the next few years. Because of their unique specificity and the fact that they can be produced in virtually unlimited quantities, monoclonal antibodies are an important tool in diagnostics. However, the use of these antibodies does not always guarantee absolute specificity, and the low affinity of many monoclonal antibodies will impose a number of limitations on their use. Monoclonal antibodies can also be used to optimize physiological processes such as growth and reproduction. For this, homologous antibodies will probably offer several advantages over their murine counterparts in terms of effectiveness for passive immunization. Some success has already been achieved in the development of monoclonal antibodies from livestock species. Finally, it is shown that monoclonal antibodies are becoming extremely powerful research tools.

### **INTRODUCTION**

When an antigen is introduced into an animal, one aspect of the immune response is the secretion by plasma cells of antibodies: immunoglobulin molecules with binding sites that recognize the shape of particular determinants on the surface of the antigen and bind to them. The combination of antibody with antigen sets in train processes that can neutralize and eliminate the antigen.

Quite apart from the natural function of antibodies in the immune response, they have long been an important tool for investigators, who capitalize on their specificity to identify or label particular molecules or cells and to separate them

from a mixture. However, the heterogeneity of the immune response has complicated the use of antibodies as reagents. If the antigen is a complex macromolecule, such as a protein, it will contain many antigenic determinants. Each of these antigenic determinants may trigger specific B-lymphocytes to differentiate and generate clones of plasma cells secreting antibody. Furthermore, a single antigenic determinant can stimulate multiple B-cell clones synthesizing antibodies that vary in specificity and affinity.

### *Conventional antiserum production*

Immunization with most antigens results in a polyclonal response and the accumulation of many different antibodies in the serum. Antisera, therefore, may contain immunoglobulins of varying isotypes, affinities, specificities and biological activities. The quantity and quality varies from animal to animal and even from one bleed to the next in a given animal. This situation is made more complex if the immunizing agent contains minor contaminants of a highly immunogenic nature that are capable of inducing large amounts of an irrelevant antibody. Finally, good antisera are usually available in limited amounts, especially if they have been raised against weak antigens or if it has been necessary to absorb the antiserum to make it specific. Consequently, the production and standardization of specific immunological reagents is a difficult task.

### *Hybridoma technology*

The hybridoma technology developed by Köhler and Milstein (1975) makes it possible to overcome these difficulties and, in many cases, to obtain a serological reagent that is not only homogeneous and predictable, but is also tailored to the needs of each of the many applications of immunological reagents (for a comprehensive review, see Milstein, 1980).

The basis of hybridoma technology is the immortalization of B-lymphocytes with antibody-producing capacities, but limited in vitro growth characteristics. The lymphocytes are fused with cells from a non-antibody-producing and continuously growing tumour cell line or myeloma cell line so that hybrids continue to secrete antibodies while gaining the immortality of the parent tumour cell. Because each B-lymphocyte produces an immunoglobulin molecule with a fixed specificity, clones derived from hybridized cell populations are homogeneous in nature, and each of the clones secretes an immunoglobulin with a single molecular structure and antigen specificity. Thus, all the antibody molecules secreted by the same cell line exhibit identical specificity, affinity and isotype (class or subclass). The antibodies produced by the fusion procedure are known as monoclonal antibodies, distinguishing them from the heterogeneous polyclonal antibodies obtained by conventional antibody production. Antibody-producing clones may be stored in liquid nitrogen in the viable state

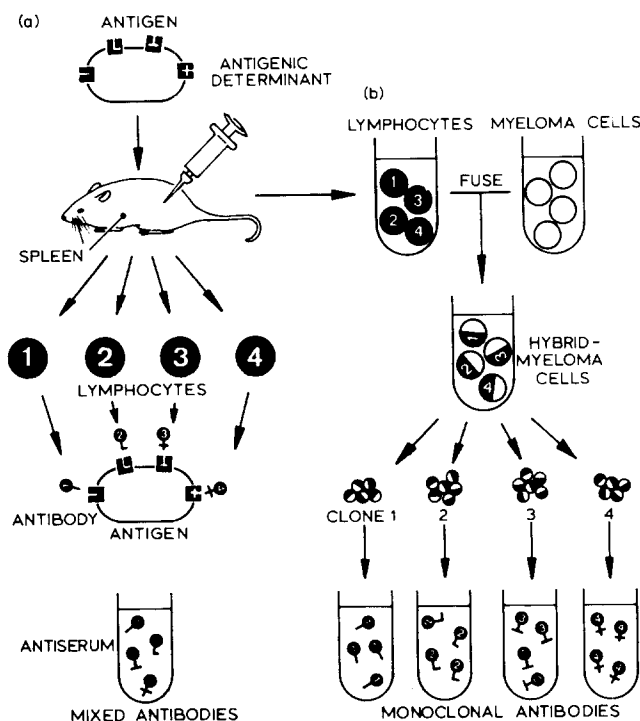


Fig. 1. Immune response is initiated (a) when an antigen molecule carrying several different antigenic determinants enters the body of an animal. The immune system responds: lines of B-lymphocytes proliferate, each secreting an immunoglobulin molecule that fits a single antigenic determinant (or a part of it). A conventional antiserum contains a mixture of these antibodies. Monoclonal antibodies are derived by fusing lymphocytes from the spleen with malignant myeloma cells (b). Individual hybrid cells are cloned, and each of the clones secretes a monoclonal antibody that specifically fits a single antigenic determinant on the antigen molecule (Milstein, 1980).

and retrieved at will for culture and production of the corresponding antibody. In Fig. 1, a comparison is made between the monoclonal antibody technique and the conventional technique to prepare antibodies.

There are a number of excellent books and reviews dealing with the specific details of monoclonal antibody production techniques (Goding, 1980, 1983; Galfrè and Milstein, 1981; Campbell, 1984). The benefits of monoclonal antibodies have resulted in the hybridoma technology being applied to many basic and practical problems. In animal production, monoclonal antibodies are increasingly finding application in the areas of diagnostics, passive immunization and fundamental research. In the following sections, some applications of monoclonal antibodies within these areas with emphasis on reproduction are discussed. A complete review is beyond the scope of this article. The aim is to describe critically the potential usefulness of monoclonal antibodies in animal

production, the inherent limitations of the current hybridoma technology and the improvements that can be foreseen within the next few years.

#### MONOCLONAL ANTIBODIES VERSUS POLYCLONAL ANTIBODIES

It is evident that monoclonal antibodies have some major advantages over conventional polyclonal antisera. Firstly, monoclonal antibodies are a homogeneous and permanently-available reagent that can be obtained in large amounts. Another major advantage is that pure antibodies can be obtained even when impure antigens were used to immunize. This is important because many biologically-significant antigens have not been purified to homogeneity. It is even possible to use hybridoma technology to look for antigens whose existence is only suspected, and whose structure and properties are completely unknown. In addition to these obvious benefits, hybridoma technology makes it possible to generate a battery of monoclonal antibodies that react with a particular antigen and to select those antibodies that will be best for a particular task. Individual monoclonal antibodies can be chosen on the basis of their affinity, specificity or isotype.

Despite the great potential of monoclonal antibodies, there are several disadvantages to their use that deserve consideration. Since a monoclonal antibody detects a single antigenic determinant it may be more influenced by conditions that alter the binding properties of the single binding site, and in addition it will not form the lattice-like antigen-antibody structure required for direct precipitation, unless the antigen has more than one identical antigenic determinant (i.e., polyvalent antigen) distributed in a favourable conformation. The utility of otherwise valuable monoclonal antibodies may also be limited by the fact that the antibodies are of a specific subclass and, therefore, restricted to the functions that can be carried out by antibodies of that class. Such limitations may be minor and can often be circumvented, but must be taken into consideration when these reagents are used in place of polyclonal antisera.

#### DIAGNOSTICS

##### *Monoclonal antibodies in diagnostics*

Several recent reviews (Scott, 1985; Sevier, 1985) have expressed the belief that monoclonal antibodies will soon replace polyclonal antisera as standard reagents for immunological assays. However, the time and effort required to obtain monoclonal antibodies with suitable properties is considerable. Furthermore, for those assays which already operate satisfactorily with adequate supplies of conventional antiserum there may be little to be gained by the production of a monoclonal antibody.

The properties of monoclonal antibodies mean that they are very valuable diagnostic tools in certain situations. Firstly, it is possible to produce specific antibodies from impure material. Secondly, for different antigens which possess common structural features, antibodies can be obtained which are directed against structures unique to a particular antigen and are, therefore, totally specific. Finally, the capacity to produce monoclonal antibodies in large quantities and with constant characteristics is valuable for assays demanding substantial amounts of antibody and for standardizing immunological assays.

### *Pregnancy diagnosis*

An example of a typical diagnostic test in animal production in which monoclonal antibodies might be used is the milk-progesterone test for confirmation of oestrus and pregnancy diagnosis in cattle (Robertson and Sarda, 1971; Heap et al., 1973). The progesterone concentration in either blood plasma or in milk at oestrus is very low and rapidly rises to a high level at mid-cycle. This level is maintained throughout pregnancy. If a cow does not become pregnant, the progesterone level falls around Day 16–19 after last oestrus. Several enzyme immunoassays for the determination of progesterone have been described in the literature, most of them employing polyclonal antibodies (Arnstadt and Cleere, 1981; Van de Wiel and Koops, 1986). However, for commercialization, the milk-progesterone test has to be standardized and in that case monoclonal antibodies are the preferred reagents. Several groups have prepared monoclonal antibodies to progesterone (Fantl et al., 1982; White et al., 1982; Booman et al., 1984a). These antibodies differed considerably in their specificity and affinity for progesterone. The best monoclonal did not detect progesterone with any greater sensitivity than the conventional polyclonal sera. However, the use of monoclonal antibodies leads to improvements in test standardization and avoids the dependency upon animals producing high quality antisera. ImmuCell Inc. (U.S.A.) has designed a rapid progesterone cow-side test based on the antibody with the best characteristics produced in our laboratory. This test can be performed by the farmer himself and the results are known within 4 min.

In pigs, increased levels of oestrone sulphate in plasma have been demonstrated early in pregnancy from Day 16 with a maximum on Day 40 (Robertson and King, 1974). A pregnancy diagnosis test can be based on the differences in plasma levels between pregnant and cyclic pigs (Guthrie and Deaver, 1979; Edqvist et al., 1980). Besides standardization of a kit for pregnancy diagnosis, monoclonal antibodies have the advantage that they can specifically bind with oestrone sulphate whereas polyclonal antibodies are mostly directed against oestrone. The necessity for hydrolysis of oestrone sulphate can, therefore, be avoided. At our laboratory very specific and high affinity monoclonal antibod-

ies have been prepared for oestrone and oestrone sulphate, respectively.

A similar application of monoclonal antibodies is their use in rapid solid-phase sandwich enzyme assays to measure equine and bovine luteinizing hormone for the detection of ovulation and equine pregnant mare serum gonadotrophin (PMSG) for pregnancy diagnosis (Kasper et al., 1985a,b; Roser et al., 1985). These monoclonal antibodies could be useful in manufacturing test kits for field applications demanding substantial amounts of antibody.

### *Sexing embryos and sperm*

Another application in animal reproduction is the use of monoclonal antibodies against the H-Y antigen for sexing bovine embryos before transplantation (for a review, see Booman, 1986). The H-Y antigen is a histocompatibility antigen, detected in 1955 by Eichwald and Silmsner, and present on the surface of male cells, but not on those of the female (Billingham and Silvers, 1960). Because of the exclusive presence of the H-Y antigen in the mammalian male (Wachtel et al., 1975) and its detection early in embryonic development (Krco and Goldberg, 1976), it has become possible to predict the phenotypic sex of the offspring on the basis of embryonic H-Y antigen expression. Polyclonal antibodies against the H-Y antigen can be raised by injecting female C57Bl/6 mice with cells of males of the same inbred strain. Anti-H-Y antibodies of the mouse have been used to identify XY cells in some 70 species from all classes of vertebrates (for references, see Wachtel, 1983). The antigen appears to lack species specificity and, therefore, the antibodies can be used to identify bovine embryos. The H-Y antigen is a weak antigen and immunization with H-Y antigen usually results in the production of low titre, low affinity antisera. Because only a low percentage of mice have a good antibody response and their sera run out quickly, it would be preferable to have monoclonal antibodies. Several monoclonal antibodies have already been developed (Koo et al., 1981; Farber et al., 1982; Shapiro and Goldberg, 1984). However, the affinity of all these monoclonal antibodies appears to be low. In our laboratory we have been trying to produce specific, high-affinity monoclonal antibodies against the H-Y antigen (Booman et al., 1988a). Evaluation of bovine embryos for expression of H-Y antigen using these monoclonal antibodies in an indirect immunofluorescence assay has been shown to be reasonably accurate. The assessment of fluorescence, however, is highly subjective. Work must still be done to modify the procedure in such a way that subjectivity is reduced.

It is unlikely that monoclonal antibodies against the H-Y antigen can be used as a means for the selection of X or Y chromosome-bearing sperm. Success would depend on haploid expression of H-Y antigen by Y-bearing spermatozoa. It is clear, however, that expression of the H-Y antigen requires the Y-linked gene as well as an X-linked one (Ohno, 1979; Wachtel and Ohno,

1980). Ohno (1982) supposed that the H-Y antigen present in abundance on the sperm plasma membrane is actually contributed by Sertoli cells and not synthesized by germ cells with a dormant X chromosome, the implication of which is that spermatozoa passively become H-Y positive regardless of sex chromosomal type.

#### *Other diagnostic applications*

A valuable in vitro diagnostic assay might be to evaluate whether sperm has been capacitated or not. Capacitation is the alteration of factors on the sperm cells necessary for fertilization (for a review, see Bedford, 1983). Monoclonal antibodies specific for appropriate surface antigens may give information about changes in surface organization that appear to accompany capacitation (Saxena et al., 1986). Preservation of boar semen during several days in appropriate buffer may not induce capacitation; on the other hand, for in vitro fertilization purposes capacitated sperm is a prerequisite. Such antibodies require a high degree of specificity.

Specificity is also of importance in the production of blood-typing reagents for farm animals. Blood group antigens have been widely used in the genetic identification of livestock, and the antigens of the major histocompatibility complex hold promise as markers for immune response genes in domestic species. Monoclonal antibodies would increase the efficiency of routine blood typing and allow for international standardization of genetic markers of many types (Tucker et al., 1981).

Another potentially valuable application is in the area of food analysis, such as the identification of the species origin of meat products and the determination of residues of hormones or growth promoters in milk and meat (for references, see Morris and Clifford, 1985). Monoclonal antibodies would improve the accuracy and reproducibility of the results from immunoassay techniques and offer a cheap standardized method of screening a large number of samples rapidly and accurately.

#### *Specificity and affinity of monoclonal antibodies*

Two remarks should be made concerning the use of monoclonal antibodies in diagnostics. In polyclonal antisera where antibodies are derived from multiple distinct B cell clones, cross-reactivity is a major problem. Although the technique of monoclonal antibody production selects for an antibody with specificity for a single antigenic determinant, this does not always preclude the presence of cross-reactivity. The specificity of an antibody is a quantitative phenomenon that is determined by its affinity for a defined antigenic structure as compared to other antigens. It is clear that the discriminating abilities of monoclonal antibodies are potentially very great, although impossible to pre-



dict in advance. The ability to obtain highly-specific antibodies obviously depends on the existence of structural features unique to a given molecule, and on the maximum affinity differences likely to be possible to allow binding to these, but not to modified determinants on other molecules. It is also necessary that such structural features make a significant contribution to the overall immunogenicity of the molecule, although even in this case it is a matter of generating enough monoclonal antibodies to find the right one.

In addition, most antibodies derived by hybridoma technology have affinities far below the corresponding conventional antisera. In a polyclonal antiserum, the majority of antibodies will also have low affinities, but the few high affinity antibodies will dominate the reaction and provide the necessary sensitivity. However, a monoclonal antibody can exhibit an affinity anywhere within the full range and one must identify those few of sufficient affinity. Some recent evidence has suggested that the high affinity of polyclonal relative to monoclonal antibodies may reflect more than a simple statistical incidence of different individual affinities in a large population. Some mixtures of 2 monoclonal antibodies for different antigenic determinants on a given antigen show co-operativity in that the affinity of the mixture is considerably higher than that of the individual antibodies (Ehrlich et al., 1983). If high affinity monoclonal antibodies are of particular importance, the screening assays for selecting relevant antibodies must be designed to exclude low affinity antibodies. All things considered, it is always necessary to evaluate carefully the need for making monoclonal instead of polyclonal antibodies for a given antigen as considerable time and effort is required to obtain monoclonal antibodies with suitable properties.

## PASSIVE IMMUNIZATION

### *Immunoneutralization by active and passive immunization*

Immunization against biologically active substances, such as hormones, can produce a wide variety of effects, including neutralization of the selected substance, blocking of action or even enhancement of action of a substance. The nature of these effects will be influenced by level, specificity and affinity of the antibodies, regardless of whether they are a result of active or of passive immunization.

It has been hypothesized (Cox et al., 1985) that in the neutralization of selected substances (the system that is most commonly considered), the active substance is bound to antibody and hence becomes biologically unavailable. In this way, there is a direct reduction of biological material available for target tissues. Many other, secondary, effects may be a consequence of the immunization, e.g., disturbance of a metabolic network and quantitative alteration in other active substances. There are now many examples showing the physio-

logical effects of immunizing animals against hormones; for instance, immunization of sheep to gonadal steroids during the breeding season may raise the ovulation rate (Pathiraja, 1982) resulting in an increased number of lambs born (Land et al., 1982). A possible mechanism that can explain such an effect is that the antisera interfere with the equilibria between gonadotrophin release and gonadal activity (Land et al., 1983). Immunization against luteinizing hormone-releasing hormone can produce immunocastration and meat quality changes in rams and bulls (Schanbacher, 1982; Robertson et al., 1982); somatostatin immunity has been reported to bring about growth changes (Spencer et al., 1983) and immunization against inhibin results in early onset of puberty as well as increased ovulation rate in sheep (O'Shea et al., 1982; Henderson et al., 1984).

Alternatively, there is a possibility that antibodies could be used effectively to bind to receptors and hence to block hormones from reaching the receptors, thus resulting in modification of hormone action.

Enhancement of action of a substance by immunization has hardly been explored, but may have very useful applications. Such effects were noted by Holder et al. (1985), who showed that the binding of a monoclonal antibody to human growth hormone resulted in marked enhancement of the somatogenic activity of the hormone *in vivo*. The same group found that the binding of some monoclonal antibodies to bovine growth hormone also resulted in enhancement of biological activity and that the effect was dependent on the binding site specificity of the monoclonal antibody (Aston et al., 1987). The mechanisms behind this phenomenon are not clear.

Immunoneutralization by active immunization is not a reproducible effect. Within species, and even within breeds, there can be marked variations in the magnitude and speed of antibody response. The effect of active immunization is manifested only in those animals which form adequate titres of antibodies. The effects could be brought about in a repeatable manner by passive administration of preformed antibodies. Also, in contrast to the slow acquisition of antibody titre following active immunization, passive immunization results in an immediate neutralization. The ability to immediately and specifically neutralize hormone activity provides a means of influencing a physiological process at a very precise moment. A good example of this is the administration of (polyclonal) antibodies against pregnant mare serum gonadotrophin (PMSG) in superovulated cattle immediately after insemination, where the surplus of PMSG is neutralized and consequently the number of embryos of good quality can increase (Bouters et al., 1983). Finally, passive immunization has proved useful in the treatment of certain animal diseases where prophylactic measures are not possible. However, the amounts of antisera required for passive immunization are very large, so that with polyclonal antibodies it is possible to treat only a restricted number of animals. The hybridoma technique offers the possibility of obtaining antibodies of the required specificity and characteristics in unlimited amounts.

The first commercial application of monoclonal antibody administration *in vivo* was to protect neonatal calves against diarrhoea caused by enterotoxigenic *Escherichia coli*. Oral administration of monoclonal antibodies specific for the K99 pilus antigen of *E. coli* passively protects animals in laboratory and field conditions (Sherman et al., 1983). Recently, a commercial preparation of monoclonal antibodies has been introduced against PMSG to improve the results of superovulation in cattle (Dieleman et al., 1988). Other applications will certainly follow.

*Limiting factors of monoclonal antibodies in passive immunization.*

It will, however, take some time for the potential of monoclonal antibodies to be fully realized in animal production. In spite of new mass culture techniques, the production costs of purified monoclonal antibodies are still high so that not all applications are yet economically attractive. Another limitation may be the reaction of the treated animal to the effects of passive immunization. For instance, in the case of anti-steroid antibodies, the animal may restore its hormone level via compensatory production, shifting of equilibria in the steroid pathway and release of steroids from fat deposits (Booman et al., 1984b; Wang et al., 1984). A third limitation might be the murine or rat origin of monoclonal antibodies when applied in other species. Once these antibodies have been recognized as foreign, an anti-mouse immunoglobulin response may develop which limits the further effectiveness of the monoclonal antibody. In addition, heterologous antibodies may not elicit cooperative cellular effects. As reported by Nose and Wigzell (1983) and reviewed by Larrick and Buck (1984), antibodies have species-specific carbohydrates which are important in several antibody effector functions.

Despite these drawbacks, murine monoclonal antibodies have been used successfully to neutralize PMSG in superovulated cattle (Dieleman et al., 1988). It is not yet known, however, whether repeated injections given over a period of time remain effective. In pigs, repeated administration of murine antibodies generated a significant antibody response to mouse immunoglobulins (Arriens and Booman, 1988). Human clinical trials with murine monoclonal antibodies indicate that almost all non-immunocompromised patients developed anti-mouse immunoglobulin antibodies which neutralized the therapeutic effect (for references, see Cole et al., 1985). The neutralizing effect of human anti-mouse antibody is due to a host immune response against both the constant portions of the mouse immunoglobulin molecule and the variable region (idiotype) reacting with the antigenic determinant (Levy et al., 1984).

These findings have important implications for the eventual use of homologous monoclonal antibodies for passive immunization. Such antibodies may likewise evoke an anti-idiotypic immune response. However, Ehrlich et al. (1987) reported that only one out of five rhesus monkeys injected repeatedly

with human monoclonal antibodies produced anti-idiotypic antibodies. They suggested that the anti-idiotypic antibodies that are prevalent in the human anti-mouse monoclonal antibody response are elicited through a hapten-like effect, in which the heterologous immunoglobulin acts as a carrier for the idiotypic.

### *Production of homologous antibodies*

Although anti-idiotypic responses cannot be excluded as a complicating factor, homologous antibodies will probably offer several advantages over their murine counterparts in terms of effectiveness for passive immunization. Unfortunately, the production of, for instance, bovine or porcine monoclonal antibodies has been greatly hampered by the lack of myeloma fusion partners for lymphocytes from these species.

Nevertheless, some success has already been achieved in the development of monoclonal antibodies from livestock species. Srikumaran et al. (1983) demonstrated the potential of interspecies hybridomas, produced by fusing mouse myeloma cells with bovine lymphocytes. This strategy circumvents the necessity of developing homologous fusion partners. More recently, the generation from such interspecies fusions of bovine antibodies with specificity for bovine enteric coronavirus (Raybould et al., 1985) as well as antigen-specific ovine monoclonal antibodies has been reported (Beh et al., 1986; Groves et al., 1987). A severe limitation to interspecies hybridomas, however, is their genetic instability, due to the selective elimination of the non-murine chromosomes.

In another approach, mouse  $\times$  bovine hybrid myelomas (heteromyelomas) have been constructed in attempts to obtain a better fusion partner for the production of bovine monoclonal antibodies (Tucker et al., 1984; Booman et al., 1988b). It was anticipated that a heteromyeloma would retain the superior fusion characteristics of the mouse myeloma cells and be better able to support stable bovine antibody production because of the presence of bovine chromosomes. With these heteromyeloma cell lines bovine antibodies have been produced against the Forssman antigen (Tucker et al., 1984) and at our laboratory against rotavirus and PMSG. Current research is focused on the effectiveness of murine monoclonal antibodies compared to bovine antibodies against PMSG in superovulated cattle after repeated treatments.

### FUNDAMENTAL RESEARCH

The potential of monoclonal antibodies as research tools is clearly enormous. This is due to the specificity of monoclonal antibodies which allows discrimination between closely related antigenic determinants.

One of the areas where monoclonal antibody technology is having a major impact is in analysis of the mechanism of immune regulation and delineation of the genetic basis of disease susceptibility. The use of monoclonal antibodies has permitted the development of precise immunological reagents that can be used to define the network of functional subpopulations of lymphoid cells in livestock species and the antigens of the major histocompatibility complex that are centrally involved in the regulation and expression of the immune response (for references, see Davis et al., 1985). Monoclonal antibodies can be applied to define the functional roles of sperm constituents during differentiation and fertilization and to identify and resolve the functions of many regulatory substances with crucial roles in mammalian gametogenesis, fertilization and development (for references, see Bellvé and Moss, 1983). A further consequence of the fact that one particular monoclonal antibody recognizes only one antigenic determinant of an antigen is that monoclonal antibodies may be useful reagents in molecular studies of receptor structure and function (Greene et al., 1980) or in studying the orientation of hormones when bound to their receptors (Moyle et al., 1982).

Monoclonal antibodies can also be used effectively as biochemical reagents for the affinity purification of antigenic molecules of interest in animal production. The affinity of monoclonal antibodies is frequently reported as being low relative to their polyclonal counterparts. Low affinity antibodies are generally more suitable than high affinity antibodies for use in immunopurification schemes (Morgan et al., 1984), because of the relative ease of elution of antigen in an active form.

A novel and useful application of monoclonal antibodies is in the development of anti-idiotypic antibodies. The binding site on the monoclonal antibody, the idiotype, consists of a region complementary to the antigenic determinant. The production of antibodies directed against another antibody molecule will result in some antibodies specific for the idiotypic region, the so-called anti-idiotypic antibodies (Marx, 1985). The binding region of the anti-idiotypic antibody mimics the architectural configuration of the original antigenic determinant. The mimicry is so accurate that in some cases the anti-idiotypic antibody may be utilized in place of antigen for the identification or induction of antibody. It has been suggested by Kelley and Lewin (1986) that this approach could be particularly useful in vaccine production for antigen preparations that might remain infectious after isolation, or for those antigens that are difficult to isolate and to characterize. Anti-idiotypic antibodies might also be used as a tool to mimic the antigen *in vitro* (e.g., diagnostic assays) or *in vivo* (e.g., to mimic insulin and cause glucose entry into a cell).

These examples are only intended to give an impression of the many possible uses of monoclonal antibodies in fundamental research and thus to show what a valuable instrument has been made available to animal sciences.

## CONCLUSIONS

The particular advantages of monoclonal antibodies can be first and most easily shown in immunodiagnosis. Once a hybridoma producing a monoclonal antibody appropriate for a particular task has been obtained, large amounts of a homogeneous and reliable reagent are available for as long as they are needed. Monoclonal antibodies may also prove to be important in passive immunization, although such applications require more basic research. The advent of better methods for producing antibodies from livestock species may increase the versatility of this technology. Finally, it is evident that monoclonal antibodies are becoming extremely powerful research tools. There is no doubt that monoclonal antibody technology will have an important impact on the improvement of animal quality and productivity.

## REFERENCES

- Arnstadt, K.I. and Cleere, W.F., 1981. Enzyme immunoassay for determination of progesterone in milk from cows. *J. Reprod. Fertil.*, 62: 173-180.
- Arriëns, M.A. and Booman, P., 1988. The effect of repeated injections of murine antibodies against progesterone on immuneresponse and plasma progesterone neutralizing efficacy in cyclic pigs. Report B-312, Research Institute for Animal Production, Zeist.
- Aston, R., Holder, A.T., Ivanyi, J. and Bomford, R., 1987. Enhancement of bovine growth hormone activity in vivo by monoclonal antibodies. *Mol. Immunol.*, 24: 143-150.
- Bedford, J.M., 1983. Significance of the need for sperm capacitation before fertilization in eutherian mammals. *Biol. Reprod.*, 28: 108-120.
- Beh, K.J., Haynes, S.E. and Ward, K.A., 1986. Characterization of two mouse myeloma  $\times$  sheep lymphocyte cell lines secreting sheep antibody. *Mol. Immunol.*, 23: 717-724.
- Bellvé, A.R. and Moss, S.B., 1983. Monoclonal antibodies as probes of reproductive mechanism. *Biol. Reprod.*, 28: 1-26.
- Billingham, R.E. and Silvers, W.K., 1960. Studies on tolerance of the Y-chromosome antigen in mice. *J. Immunol.*, 85: 14-26.
- Booman, P., 1986. Control of sex ratio by sexing sperm and embryos. In: C. Smith, J.W.B. King and J.C. McKay (Editors), *Exploiting New Technologies in Animal Breeding*. Genetic Developments. Oxford University Press, Oxford, pp. 13-22.
- Booman, P., Tieman, M., van de Wiel, D.F.M., Schakenraad, J.M. and Koops, W., 1984a. Application of monoclonal antibodies in animal production: pregnancy diagnosis in cattle. In: E.H. Houwink and R.R. van der Meer (Editors), *Innovations in Biotechnology*. Elsevier, Amsterdam, pp. 259-266.
- Booman, P., van de Wiel, D.F.M., Schakenraad, J.M., Koops, W., Tieman, M. and Kruijt, L., 1984b. Administration of monoclonal antibodies against progesterone in cyclic pigs. *Proceedings of the 10th International Congress on Animal Reproduction and Artificial Insemination, Illinois, III*: 503-505.
- Booman, P., Kruijt, L., Tieman, M., Piedrahita, J.A., Veerhuis, R., de Boer, P. and Ruch, F.E., 1988a. Production and characterization of monoclonal antibodies against the H-Y antigen. *J. Reprod. Immunol.*, submitted.
- Booman, P., Tieman, M., van Zaane, D., Bosma, A.A. and de Boer, G.F., 1988b. Construction of

- a bovine-murine heteromyeloma cell-line; production of bovine monoclonal antibodies against rotavirus and pregnant mare serum gonadotrophin. *Vet. Immunol. Immunopathol.*, submitted.
- Bouters, R., Moyaert, I., Coryn, M. and Vandeplasse, M., 1983. The use of a PMSG antiserum in superovulated cattle: endocrinological changes and effects on timing of ovulation. *Zucht-hygiene*, 18: 172-177.
- Campbell, A.M., 1984. *Monoclonal Antibody Technology*. Elsevier, Amsterdam.
- Cole, S.P.C., Kozbor, D. and Roder, J.C.C., 1985. Strategies for production of human monoclonal antibodies. In: T.A. Springer (Editor), *Hybridoma Technology in the Biosciences and Medicine*. Plenum Press, New York, pp. 43-55.
- Cox, R.I., Wilson, P.A. and Wong, M.S.F., 1985. Manipulation of endocrine systems through the stimulation of specific immune responses. In: R.A. Lang, J.S.F. Barker, D.B. Adams and K.J. Hutchinson (Editors), *Reviews in Rural Science*, 6. Armidale, University of New England, pp. 150-160.
- Davis, W.C., Yilma, T., Perryman, L.E. and McGuire, T.C., 1985. Perspectives on the application of monoclonal antibody and transfection technology in veterinary microbiology. *Prog. Vet. Microbiol. Immunol.*, 1: 1-24.
- Dieleman, S.J., Bevers, M.M., Wurth, Y.A., Gielen, J.Th. and Willemse, A.H., 1988. Improved embryo yield and donor ovaries in cows after PMSG-superovulation with monoclonal anti-PMSG administered shortly after the preovulatory LH peak. *Theriogenology*, in press.
- Edqvist, L.E., Einarsson, S. and Larsson, K., 1980. Early pregnancy diagnosis in pigs by assay of oestrone sulphate in peripheral blood plasma. *Proceedings of the International Pig Veterinary Society Congress*, Copenhagen, 27 pp.
- Ehrlich, P.H., Moyle, W.R. and Moustafa, Z.A., 1983. Further characterization of cooperative interactions of monoclonal antibodies. *J. Immunol.*, 131: 1906-1912.
- Ehrlich, P.H., Harfeldt, K.E., Justice, J.C., Moustafa, Z.A. and Östberg, L., 1987. Rhesus monkey responses to multiple injections of human monoclonal antibodies. *Hybridoma*, 6: 151-160.
- Eichwald, E.J. and Silmsen, C.R., 1955. Untitled communication. *Transplant. Bull.*, 2: 148-149.
- Fantl, V.E., Wang, D.Y. and Knyba, R.E., 1982. The production of high affinity monoclonal antibodies to progesterone. *J. Steroid Biochem.*, 17: 125-130.
- Farber, C.M., Wachtel, S.S. and Cunningham-Rundles, C., 1982. Immune complexes containing H-Y antigen and maternal IgG in cord serum. *Clin. Exp. Immunol.*, 50: 450-453.
- Galfrè, G. and Milstein, C., 1981. Preparation of monoclonal antibodies: strategies and procedures. In: J.J. Langone and H. Van Vunakis (Editors), *Methods in Enzymology*. Academic Press, London, Vol. 73, pp. 3-46.
- Goding, J.W., 1980. Antibody production by hybridomas. *J. Immunol. Methods*, 39: 285-308.
- Goding, J.W., 1983. *Monoclonal Antibodies: Principles and Practice*. Academic Press, London.
- Greene, G.L., Fitch, F.W. and Jensen, E.V., 1980. Monoclonal antibodies to estrophilin: probes for the study of estrogen receptors. *Proc. Natl. Acad. Sci., U.S.A.*, 77: 157-161.
- Groves, D.J., Morris, B.A., Tan, K., De Silva, M. and Clayton, J., 1987. Production of an ovine monoclonal antibody to testosterone by an interspecies fusion. *Hybridoma*, 6: 71-76.
- Guthrie, H.D. and Deaver, D.R., 1979. Estrone concentration in the peripheral plasma of pregnant and non-pregnant gilts. *Theriogenology*, 11: 321-329.
- Heap, R.B., Gwyn, M., Laing, J.A. and Walters, D.E., 1973. Pregnancy diagnosis in the cow; changes in milk progesterone concentration during the oestrous cycle and pregnancy measured by rapid radioimmunoassay. *J. Agric. Sci.*, 81: 151-157.
- Henderson, K.M., Franchimont, P., Lecomte-Yerna, M.J., Hudson, N. and Ball, K., 1984. Increase in ovulation rate after active immunization of sheep with inhibin partially purified from bovine follicular fluid. *J. Endocrinol.* 102: 305-309.
- Holder, A.T., Aston, R., Preece, M.A. and Ivanyi, J., 1985. Monoclonal antibody-mediated enhancement of growth hormone activity in vivo. *J. Endocrinol.*, 107: R9-R12.

- Kasper, K.C., Marr, G. and Fashandi, S., 1985a. Field dipstick ELISA for PMSG and equine LH. *Biol. Reprod.*, 32 (Suppl. 1): 223.
- Kasper, K.C., Marr, G. and Fashandi, S., 1985b. The DiaSTICK PMSG test: performance characteristics and clinical evaluation. *J. Anim. Sci.*, 61 (Suppl. 1): 389-390.
- Kelley, K.W. and Lewin, H.A., 1986. Monoclonal antibodies: pragmatic application of immunology and cell biology. *J. Anim. Sci.*, 63: 288-309.
- Köhler, G. and Milstein, C., 1975. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature (London)*, 256: 495-497.
- Koo, G.C., Tada, N. and Hämmerling U., 1981. Monoclonal anti-H-Y antibodies. In: G.J. Hämmerling, U. Hämmerling and J.F. Kearney (Editors), *Monoclonal Antibodies and T-Cell Hybridomas. Perspectives and Technical Advances*. Elsevier/North-Holland Biomedical Press, Amsterdam, pp. 143-147.
- Krcó, C.J. and Goldberg, E.H., 1976. Detection of H-Y (male) antigen on 8-cell mouse embryos. *Science*, 193: 1134-1135.
- Land, R.B., Morris, B.A., Baxter, G., Fordyce, M. and Forster, J., 1982. Improvement of sheep fecundity by treatment with antisera to gonadal steroids. *J. Reprod. Fertil.*, 66: 625-634.
- Land, R.B., Fordyce, M., Gauld, I.K., Morris, B.A. and Webb, R., 1983. Fertility of sheep given antisera to steroids during anoestrus. *J. Reprod. Fertil.*, 67: 269-273.
- Larrick, J.W. and Buck, D.W., 1984. Practical aspects of human monoclonal antibody production. *Bio Techniques*, 1: 6-14.
- Levy, R., Stratte, P., Link, M., Maloney, D.G., Oseroff, A. and Miller, R.A., 1984. Monoclonal antibodies to human lymphocytes. In: R.H. Kennett, K.B. Bechtol and T.J. McKearn (Editors), *Monoclonal Antibodies and Functional Cell Lines. Progress and Applications*. Plenum Press, New York, pp. 193-214.
- Marx, J.L., 1985. Making antibodies without the antigens. *Science*, 228: 162-165.
- Milstein, C., 1980. Monoclonal antibodies. *Sci. Am.*, 243: 56-64.
- Morgan, B.P., Daw, R.A., Siddle, K., Luzio, J.P. and Campbell, A.K., 1984. Immunoaffinity purification of human complement C9 using monoclonal antibodies. *J. Immunol. Methods*, 64: 269-281.
- Morris, B.A. and Clifford, M.N., 1985. *Immunoassays in food analysis*. Elsevier, Amsterdam.
- Moyle, W.R., Ehrlich, P.H. and Canfield, R.E., 1982. Use of monoclonal antibodies to subunits of human chorionic gonadotropin to examine the orientation of the hormone in its complex with receptor. *Proc. Natl. Acad. Sci., U.S.A.*, 79: 2245-2249.
- Nose, M. and Wigzell, H., 1983. Biological significance of carbohydrate chains on monoclonal antibodies. *Proc. Natl. Acad. Sci., U.S.A.*, 80: 6632-6636.
- Ohno, S., 1979. *Major Sex-Determining Genes*. Springer-Verlag, Berlin.
- Ohno, S., 1982. Expression of X- and Y-linked genes during mammalian spermatogenesis. In: R.P. Amann and G.E. Seidel, Jr. (Editors), *Prospects for Sexing Mammalian Sperm*. Colorado Associated University Press, Boulder, CO, pp. 109-113.
- O'Shea, T., Cummins, L.J., Bindon, B.M. and Findlay, J.K., 1982. Increased ovulation rate in ewes vaccinated with an inhibin-enriched fraction from bovine follicular fluid. *Proc. Aust. Soc. Reprod. Biol.*, 14: 85.
- Pathiraja, N., 1982. Physiological basis of genetic variation in ovulation rate. Ph.D. thesis, University of Edinburgh.
- Raybould, T.J.G., Crouch, C.F., McDougall, L.J. and Watts, T.C., 1985. Bovine-murine hybridoma that secretes bovine monoclonal antibody of defined specificity. *Am. J. Vet. Res.*, 46: 426-427.
- Robertson, H.A. and Sarda, I.R., 1971. A very early pregnancy test for mammals: its application to the cow, ewe and sow. *J. Endocrinol.*, 49: 407-419.
- Robertson, H.A. and King, G.J., 1974. Plasma concentrations of progesterone, oestrone, oestra-



- diol-17 $\beta$  and of oestrone sulphate in the pig at implantation, during pregnancy and at parturition. *J. Reprod. Fertil.*, 40: 133-141.
- Robertson, I.S., Fraser, H.M., Innes, G.M. and Jones, A.S., 1982. Effect of immunological castration on sexual and production characteristics in male cattle. *Vet. Rec.*, 111: 529-531.
- Roser, J.F., Ruth, M., Schoenemann, H.M., Mathison, B.D. and Reeves, J.J., 1985. A rapid, sensitive monoclonal antibody based bovine luteinizing hormone test for detection of ovulation in cows. *J. Anim. Sci.*, 61 (Suppl. 1): 389.
- Saxena, N., Peterson, R.N., Sharif, S., Saxena, N.K. and Russell, L.D., 1986. Changes in the organization of surface antigens during in-vitro capacitation of boar spermatozoa as detected by monoclonal antibodies. *J. Reprod. Fertil.*, 78: 601-614.
- Schanbacher, B.D., 1982. Responses of ram lambs to active immunization against testosterone and luteinizing hormone-releasing hormone. *Am. J. Physiol.*, 242 (Endocrinol. Metab. 5): E201-E205.
- Scott, M.G., 1985. Monoclonal antibodies - approaching adolescence in diagnostic immunoassays. *Trends Biotechnol.*, 3: 170-175.
- Sevier, E.D., 1985. Monoclonal antibodies in diagnostic assay systems. *J. Med. Technol.*, 2: 287-293.
- Shapiro, M. and Goldberg, E.H., 1984. Analysis of a serological determinant of H-Y antigen: evidence for carbohydrate specificity using an H-Y specific monoclonal antibody. *J. Immunogen.*, 11: 209-218.
- Sherman, D.M., Acres, S.D., Sadowski, P.L., Springer, J.A., Bray, B., Raybould, T.J.G. and Muscoplat, C.C., 1983. Protection of calves against fatal enteric colibacillosis by orally administered *Escherichia coli* K99-specific monoclonal antibody. *Infect. Immun.*, 42: 653-658.
- Spencer, G.S.G., Garssen, G.J. and Hart, I.C., 1983. A novel approach to growth promotion using auto-immunization against somatostatin. I. Effects on growth and hormone levels in lambs. *Livest. Prod. Sci.*, 10: 25-37.
- Srikumaran, S., Guidry, A.J. and Goldsby, R.A., 1983. Bovine  $\times$  mouse hybridomas that secrete bovine immunoglobulin G1. *Science*, 220: 522-524.
- Tucker, E.M., Wright, L.J. and Varden, S.F., 1981. Monoclonal antibodies to blood group antigens on ruminant red cells. *Immunol. Lett.*, 3: 121-123.
- Tucker, E.M., Dain, A.R., Clarke, S.W. and Donker, R.A., 1984. Specific bovine monoclonal antibody produced by a re-fused mouse/calf hybridoma. *Hybridoma*, 3: 171-176.
- Van de Wiel, D.F.M. and Koops, W., 1986. Development and validation of an enzyme immunoassay for progesterone in bovine milk or blood plasma. *Anim. Reprod. Sci.*, 10: 201-213.
- Wachtel, S.S., 1983. H-Y Antigen and the Biology of Sex Determination. Grune and Stratton, New York.
- Wachtel, S.S. and Ohno, S., 1980. The immunogenetics of sexual development. *Annu. Rev. Med. Genet.*, 3: 109-142.
- Wachtel, S.S., Koo, G.C. and Boyse, E.A., 1975. Evolutionary conservation of H-Y (male) antigen. *Nature (London)*, 254: 270-272.
- Wang, M.-Y., Rider, V., Heap, R.B. and Feinstein, A., 1984. Action of antiprogestosterone monoclonal antibody in blocking pregnancy after postcoital administration in mice. *J. Endocrinol.*, 101: 95-100.
- White, A., Anderson, D.C. and Daly, J.R., 1982. Production of a highly specific monoclonal antibody to progesterone. *J. Clin. Endocrinol. Metab.*, 54: 205-207.

## RESUME

Booman, P., 1988. Application des anticorps monoclonaux dans la production animale: une revue. *Livest. Prod. Sci.*, 18: 119-215 (en anglais).

La technologie des hybridomes pour produire des anticorps monoclonaux évite la plupart des contraintes associées à l'utilisation des antisérums conventionnels. Cela élargit en conséquence l'aire des applications des anticorps dans les sciences animales. Dans cette mise au point on discute l'utilité potentielle des anticorps monoclonaux dans la production animale en mettant l'accent sur la reproduction, y compris les limites inhérentes de la technologie actuelle et les améliorations qui peuvent être envisagées pour les prochaines années. A cause de leur spécificité unique, et du fait qu'ils peuvent être produits en quantité virtuellement illimitée, les anticorps monoclonaux sont un important outil diagnostique. Cependant, leur utilisation ne garantit pas toujours une spécificité absolue et la faible affinité de nombre d'entre eux en limitera l'emploi. Les anticorps monoclonaux peuvent être aussi utilisés pour optimiser les processus physiologiques tels que la croissance ou la reproduction. Pour cela, les anticorps homologues présenteront probablement plusieurs avantages sur leurs contreparties murines en ce qui concerne leur efficacité pour l'immunisation passive. On a déjà obtenu un certain succès dans le développement des anticorps monoclonaux pour le bétail. On montre enfin que les anticorps monoclonaux sont des outils de recherche extrêmement puissants.

## KURZFASSUNG

Booman, P., 1988. Anwendung monoklonaler Antikörper in der Tierproduktion; ein Übersichtsreferat. *Livest. Prod. Sci.*, 18: 119-215 (auf englisch).

Die Hybridisierungstechnik zur Produktion monoklonaler Antikörper umgeht viele der Beschränkungen, die bei der Verwendung konventioneller Antiseren auftreten, und erweitert dementsprechend die Anwendungsgebiete von Antikörpern in den Tierzuchtwissenschaften. In der vorliegenden Übersicht wird der mögliche Nutzen monoklonaler Antikörper in der Tierproduktion, mit besonderer Berücksichtigung der Reproduktion, diskutiert, einschließlich der Begrenzungen der gegenwärtigen Technologie und den in den nächsten Jahren voraussehbaren Verbesserungen. Aufgrund ihrer einzigartigen Spezifität und aufgrund der Tatsache, daß sie in praktisch unbegrenzter Menge hergestellt werden können, sind monoklonale Antikörper ein wichtiges Hilfsmittel in der Diagnostik. Allerdings garantiert der Gebrauch dieser Antikörper nicht in allen Fällen absolute Spezifität, und die geringe Affinität vieler monoklonaler Antikörper bedingt eine Reihe von Anwendungseinschränkungen. Monoklonale Antikörper können auch zur Optimierung physiologischer Prozesse wie z.B. Wachstum und Reproduktion verwendet werden. Dazu bieten wahrscheinlich homologen Antikörper verschiedene Vorteile gegenüber murinen Antikörper, was die Effektivität zur passiven Immunisierung betrifft. Es sind schon einige Erfolge bei der Entwicklung monoklonaler Antikörper von Nutztieren erzielt worden. Abschließend wird darauf hingewiesen, daß monoklonale Antikörper äußerst wirkungsvolle Hilfsmittel für die Wissenschaft darstellen.