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The Production and Application of Non-Rodent Monoclonal Antibodies in Veterinary Science

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ABSTRACT

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The requirement for monoclonal antibodies derived from species other than rats and mice is becoming increasingly realised in veterinary, as well as human, medicine. This paper reviews current knowledge of the production of inter-species hybridomas (heterohybridomas) by the fusion of rodent myeloma cell lines with lymphocytes from species of veterinary importance. To date a number of monoclonal immunoglobulins derived from sheep, cattle, pig, rabbit, mink and primate species have been produced to a variety of different bacterial, viral and nematode pathogens as well as to blood group and MHC determinants and to hormones. The technique opens up a number of possibilities for the future; some of these applications are discussed in relation to the antibodies produced thus far.

INTRODUCTION

Since the production of monoclonal antibodies (mAb) was first described by Köhler and Milstein (1975), their use has spread to nearly every field of the biological and medical sciences. Most of the mAb described to date have been of rodent origin, but there are many instances where mAb derived from other species would be advantageous. One such use would be the administration of mAb for therapeutic or prophylactic purposes where rodent mAb would evoke an immune reaction in the recipient (see review by Chatenoud, 1986). Other instances are in parasitology when mAb are required to identify host-specific, protective, antigens of a parasite (Kennedy et al., 1988; Anderson et al., 1989); in the production of anti-idiotypes (Sacks, 1987) or DNA cloning expression systems (Udomsangpetch et al., 1986). In studies of species-specific immunoglobulin (Ig) structure and organisation mAb derived from the

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TABLE 1

Details of inter-specific fusions

Species	Myeloma	Antigen	Lymphocyte source	Fusion time after 'boost'	Ig class	Minimum length of secretion	Affinity	Reference
Sheep	NS1	sheep red blood cells	spleen popliteal LN	15, 3 days (i.m. and s.c.)	surface Ig	-	-	Tucker et al., 1981
Sheep	Mouse	luteinising hormone	PBL	-	Ig	several months	-	Dicker et al., 1985
Sheep	SP2	azo-benzine arsonate-ovalbumin	popliteal LN	4 days (s.c.)	IgG	16 months	$3\times10^{8}M^{-1}$	Beh et al., 1986
Sheep	NS1×s	sheep red blood cells	popliteal LN	3–4 days (i.m.)	IgG	several months	-	Anderson et al., 1986
Sheep	NS1	testosterone-3CMO-ovalbumin	PBL	10, 5 days (i.m.)	IgG1	6 months	$7.63\times10^{-12}M$	Groves et al., 1987a
Sheep	NSO	Ostertajia	PBL	5 days larval dose	IgG	several months	-	Anderson et al., 1989
Sheep	NS1	Progesterone-11α hemisuccinate ovalbumin	PBL	-	IgG1	-	-	Groves et al., 1988b
Cattle	NS1 P3X63	bovine leukaemia virus (infected)	PBL	not immunised	IgM	-	-	Davidson et al., 1982
Cattle	SP2	-	spleen	-	IgG1 IgG2, IgM	16 months	-	Srikumaran et al., 1982, 1984
Cattle	NSO×c	sheep red blood cells	popliteal LN	3 days (i.m. and s.c.)	IgG1	several years	-	Tucker et al., 1984
Cattle	SP2	DMP-ovalbumin	spleen	3 day i.v.	Ig	-	-	Guidry et al., 1986
Cattle	SP2	bovine enteric coronavirus	spleen	-	IgG2	3 months	-	Raybould et al., 1985b
Cattle	NSO×c NSO×c×c	sheep and cattle red blood cells	popliteal LN	3–4 days (i.m.)	IgG1 IgM	-	-	Anderson et al., 1986
Cattle	SP2	heat-killed streptococcus	mesenteric/supra-mammary LN	4 days (i. mammary)	-	-	-	Guidry et al., 1986
Cattle	NSO×c×c	cattle red blood cells	popliteal LN	3–4 days (i.m. and s.c.)	IgG1 IgM	several months	-	Tucker et al., 1987

Cattle	NSO NSO×c NSO×c×c	*E. coli* k99 pilus antigen	popliteal LN	3–4 days (i.m. and s.c.)	IgG1	several years	-	Anderson et al., 1987
Cattle	NS1×c	testosterone-3CMO-ovalbumin	PBL	5 days (i.m.)	IgG	25 months	$2.5\times10^{-11}M$	Groves et al., 1987b
Cattle	NS1	foot and mouth disease virus	prescapular LN	3 days (i. lymph node)	-	6 months	-	Butcher, 1987
Cattle	NS1×c	oestradiol-6CMO-ovalbumin	PBL	3 days (i.m.)	IgG1	20 months	$5.33\times10^{-10}M$	Groves et al., 1988a
Cattle	NS1 NSO×c×c	bovine respiratory syncytial virus	prescapular LN	4, 3, 2 days (s.c.)	IgG1 IgG2	several months	-	Kennedy et al., 1988
Cattle	NSO×c	cattle lymphocytes	popliteal LN	4 days (i.m. and s.c.)	IgG1	-	-	Kemp et al., 1989
Pig	SP2	*E. coli*	spleen	-	IgG	4 months	-	Raybould et al., 1985a
Rabbit	P3X63 NS1 SP2	streptococcal C vaccine	spleen/LN	-	Ig chains	several months	-	Yarmush et al., 1980
Rabbit	P3X63	streptococcal C vaccine	spleen	-	IgG, IgM	several months	-	Kuo et al., 1985
Rabbit	SP2	group A streptococcus	spleen	-	IgG, IgM	4 months	-	Raybould and Takahashi, 1988
Rhesus monkey	SPC-H20	OKT4	PBL	3 days (i.v.)	Ig	6 months	-	Van Meurs and Jonker, 1986
Monkey	P3X63	plasmodium infected human rbc	spleen PBL	3 days (i.p.) in vitro stim. 4 days	Ig	several months	-	Stanley and Reese, 1985
Chimpanzee	SPC-H20	OKT4	PBL	3 days (i.v.)	Ig	6 months	-	Van Meurs and Jonker, 1986
Mink	P3X63 SP2 NSO	mink IgG	spleen	3–4 days	Ig	-	-	Galakhar et al., 1988

Myeloma lines: NS1=NS1/1.Ag 4.1; NSO=derived from NS1; SP2=SP2/0.Ag 1.4; P3X63=P3/X63 Ag.8; SPC-H20=mouse × human.
LN=lymph node; PBL=peripheral blood lymphocytes; i.m.=intramuscular injection; i.v.=intravenous injection; s.c.=subcutaneous injection.
c=cattle; s=sheep.

relevant species are necessary. Such mAb can also be used for the production of antisera to isotypic determinants as well as for the provision of serological standards (Srikumaran et al., 1987). Moreover, the isolation of cell lines producing Igs makes it possible to clone the relevant genes and study their transcription and translation (Buckel et al., 1987). Species-specific carbohydrate groups on Igs have been shown to be important in several antibody effector mechanisms. It is therefore possible that rodent mAb may not execute effector functions such as complement fixation or Fc binding when used in non-rodent species (Nose and Wigzell, 1983). Finally, it has often proved difficult to make mouse mAb which recognise the allelic differences that exist within other species. In cross-species immunisations the overwhelming response will be to the heterologous antigens, and antibodies to the more 'subtle' polymorphic determinants such as blood group or MHC determinants may not be detected (Tucker et al., 1987; Kemp et al., 1989).

In human medicine much research is being conducted on the production of human mAb (see James and Bell, 1987). In parallel with these studies, investigations into the production of mAb derived from economically important animal species have also been initiated. The purpose of this review is to bring together the scattered literature on this subject, to assess the various methodological approaches that have been used to date, and to discuss future possibilities in the field of veterinary medicine.

IMMUNIZATION PROTOCOLS AND SOURCE OF LYMPHOCYTES FOR FUSION

In establishing the optimum conditions for production of any mAb, it is necessary to consider the nature of the immunogen, the immunisation protocol, the source of lymphocytes, and the time interval between immunisation and collection.

By using domestic animals as a source of lymphocytes it is possible to employ a variety of immunisation procedures, and to remove spleen or lymph nodes by surgery or, if necessary, after slaughter of the animal. Peripheral blood lymphocytes can also be readily obtained. Consequently there is more scope for experimentation than with human mAb production. Table 1 shows the procedures that have been used to date in different species. Lymphocytes for fusion have been successfully recovered from a variety of sources: from local secondary stimulation of a lymph node (Anderson et al., 1987; Tucker et al., 1987; Kennedy et al., 1988); from peripheral blood (Groves et al., 1987a, b, 1988a); and from spleen (Srikumaran et al., 1984; Raybould et al., 1985a, b).

In general, the requirements for immunisation to produce mAb are the same as for polyclonal antibodies and will depend on individual circumstances. The timing of the fusion following a booster dose is of prime importance and may well vary between individuals and species. Where comparative studies have been made in the human field, the optimum time for harvesting peripheral

blood lymphocytes was considered to be 6–7 days (Bogard et al., 1985) or 3 days for spleen lymphocytes (Schwaber et al., 1984). Table 1 shows the timings used for the production of non-rodent mAb of veterinary interest. The most usual time is 3–4 days post booster dose. In cattle a suitable immunisation schedule is to prime intramuscularly with antigen and Freund's complete adjuvant, and to boost after a delay of at least 3 weeks with antigen in incomplete adjuvant, injected subcutaneously into the draining lymph node. Three to 4 days later the lymph node is removed, i.e. at a time when the antibody titre is rising but has not yet reached its peak (Anderson et al., 1987). When cattle or sheep peripheral blood is used as lymphocyte source, collection of lymphocytes 3–5 days post boost has been found to be most successful (Groves et al., 1987b).

MYELOMA FUSION PARTNERS

Ideally, myeloma cell lines derived from the species of interest would be the fusion partners of choice for the production of non-rodent mAb. However, no such lines are currently available although lymphoblastomas are found in domestic species such as horses, cats and cattle. To date, therefore, attention has been directed towards the construction and use of heterohybridomas for producing mAb of veterinary interest, i.e. to fuse rodent myeloma cells with lymphocytes from immunised farm or domestic animals.

A major problem encountered with such heterohybridomas is that although they usually grow well in culture they are, like all inter-specific somatic cell hybrids, chromosomally unstable and lose most of the non-rodent chromosomes as they divide (Weiss and Green, 1967). Even if the heterohybridomas initially secrete the desired antibody they will therefore cease to do so if they lose the relevant chromosomes. Nevertheless stable antibody-secreting lines can be produced by this method and at present all the non-rodent monoclonal antibodies of interest to veterinary science have been produced in this way (Table 1).

In attempts to improve stability, several groups have used heterohybridoma cell lines as fusion partners in place of mouse or rat myelomas (Table 1). Such fusion partners must first be selected for aminopterin sensitivity; this is achieved by passaging the cells for several months in the presence of 20 μg/ml of 8-azaguanine (Littlefield, 1964) or, to hasten the process, by cloning the hybridomas on soft agar in the presence of 8-azaguanine (Tucker et al., 1984, 1987). The technique has been taken further in that as well as 'primary' (mouse × other species (sp.)) heterohybridomas, 'secondary' ((mouse × sp.) × sp.) and even 'tertiary' ((mouse × sp. × sp) × sp.) cell lines have been constructed with the aim of increasing the number of non-rodent chromosomes retained. This re-fusion procedure resulted in an increase in bovine chromosomes from 7–8% in 'primary', to 14% in 'secondary' and 18–19% in

'tertiary' heterohybridomas (Anderson et al., 1987). However, there was a concomitant increase in the number of polyploid cells and the cell population became increasingly heterogeneous in size. Results demonstrate that at least in cattle (Anderson et al., 1987; Groves et al., 1987b; Tucker et al., 1987), and possibly sheep (Anderson et al., 1986; Groves et al., 1987c), it is beneficial to use a 'primary' or 'secondary' heterohybridoma fusion partner rather than a mouse or rat myeloma cell line. It is doubtful whether the benefits continue to accrue beyond this point.

The first rodent myeloma cells used for hybridoma studies were secretors of immunoglobulins. The resultant hybridomas could potentially produce mixed Ig chains derived from both parental lines, thus reducing the chances of isolating an antibody molecule composed of the heavy and light chains of the lymphocyte Ig (Köhler et al., 1978). Later, the availability of myelomas which neither synthesised nor secreted Ig overcame this problem (Schulman et al., 1978). The same arguments apply to the choice of fusion partners for the production of heterohybridomas where it is especially important that no rodent Ig chains are secreted.

FUSION

All reported inter-specific fusions to date have used polyethylene glycol (PEG) as a fusing agent. PEG 1500 is routinely used, although PEG 4000 has also been shown to be satisfactory. Fusion techniques used in heterohybridoma work are basically modifications of those developed for murine fusions. It has been suggested (Sogn, 1987) that increased exposure time to PEG may be advantageous in rabbit × mouse fusions. However, the cytotoxicity of PEG dictates that exposure time should be kept to a minimum compatible with effective fusion. In our experience a 2 min exposure to 50% PEG gives good results. Most workers now plate out fused cells directly into HAT medium containing 10 or 20% serum.

Whilst some laboratories make use of feeder cells in their heterohybridoma culture systems (Yarmush et al., 1980; Guidry et al., 1986; Van Meurs and Jonkers, 1986; Groves et al., 1987b), others have not found it necessary to do so (Beh et al., 1986; Tucker et al., 1987). The use of feeder cells following fusion will depend on the dilution at which the fused cells are plated out, since at higher concentrations, those which are not fused will initially act as feeders.

CULTURE TECHNIQUES

Culture conditions used for the production of heterohybridomas are the same as those used for normal hybridomas, but because of their inherent instability it is wise to fuse larger numbers of cells and set up more culture plates than for mouse × mouse fusions. Base media are either RPMI (Van Meurs and Jonker, 1986; Tucker et al., 1987; Anderson et al., 1987), DMEM (Yarmush et al., 1980;

Davidson et al., 1982; Groves et al., 1988a) or a 50:50 mixture of the two (Srikumaran et al., 1984). The basal medium is supplemented with 10 to 20% serum. The use of rabbit serum in the production of rabbit × mouse heterohybridomas has been reported to improve the yield of stable antibody-secreting clones (Raybould and Takahashi, 1988) and this may apply to other species. The choice of serum may also be influenced by the screening assay. Typical serum-supplemented heterohybridoma cell supernatant contains less than 20 μg/ml of specific antibody against a background of 4–4.5 mg/ml of serum proteins. Most bovine and ovine heterohybridomas can be readily adapted to a variety of different sera or serum-substitutes as well as several commercially available serum-free preparations (Groves et al., 1987c; Groves, unpublished data, 1988). These latter media exhibit earlier signs of exhaustion compared to serum-supplemented media and antibody is often produced at a lower rate.

Fusion efficiencies in inter-species fusion are similar to those obtained in murine fusions. However, the greater genetic disruption of inter-species, compared to intra-species, fusions results in a frequently reported poor stability of lines generated. Because of this, it is important to clone as soon as possible after the identification of specific antibody in the culture supernatants. The first cloning stage often exhibits very low efficiency and it can be useful to try cloning at high cell concentrations initially, followed by a secondary cloning if monoclonality is not assured. Subsequent cloning stages should maintain $>80\%$ antibody positive lines. Careful monitoring of cultures over the first months should reveal any decline in antibody secretion rates indicative of the necessity to reclone. Loss of secreting clones during the first 3 weeks of a fusion can be fairly dramatic, although some antibody detected at early stages may be the result of antibody production by residual unfused lymphocytes. Once stability of the cell line has been established during these early stages, the line will generally prove to be as robust as any murine hybridoma in culture or storage.

SCREENING ASSAYS

The choice of screening assays selected for inter-specific fusion work necessarily varies with the desired end product. Studies of the fusion process itself, the preparation of Ig isotype standards, or situations where a rapid reliable specific antibody assay is not available, require broad Ig assays such as those described by Srikumaran et al. (1984) and Tucker et al. (1984). In all cases it is useful to screen using a system which does not cross-react either with murine immunoglobulins, to avoid expending effort on possible revertant mouse lines (Davidson et al., 1982), or with immunoglobulins present in the supplementing serum. For example fetal calf serum, because of its endogenous Ig level, interferes with assays for monoclonal cattle and sheep Igs; in such cases horse serum can be substituted (Anderson et al., 1987). This interference problem can also be reduced or eliminated by using assay reagent antibodies raised in

the same species as that providing the medium supplementing serum (Groves and Clayton, unpublished data, 1987).

ANTIBODY CHARACTERISATION

Determination of the class of non-murine antibodies is restricted by the lack of availability of class and subclass antibodies of reliable quality. This situation will improve as inter-specific fusion technology is used to create libraries of monoclonal immunoglobulins for use as standards and in the preparation of reagents (Srikumaran et al., 1987). Table 1 lists the Ig class of mAb produced to date.

ASCITES PRODUCTION

Heterohybridoma cell lines cannot readily be propagated in vivo using untreated normal mouse strains. Tumours failed to become established in Balb/c mice following the injection of mouse×human (Ostberg and Pursch, 1983) or mouse×cattle (Kennedy et al., 1988; Groves and Tucker, unpublished observations, 1988) heterohybridoma cells. Cells are probably rejected because they express some cell surface antigens from the non-murine partner.

Successful results have been obtained using Balb/c genetically athymic (nude) mice with mouse×human (Abrams et al., 1984), mouse×cattle (Groves et al., 1987c; Kennedy et al., 1988) and mouse×rabbit (Sogn, 1987) hybrids. Irradiated, chemically immunosuppressed (Weissman et al., 1985; Ostberg, 1986) or SCID mice (Ware et al., 1985) could also be used.

Raybould et al. (1985a, b) have suggested the use of suitably immunosuppressed calves or pigs for the production of large volumes of ascites containing antibodies derived from these species. Initial attempts to grow mouse×cattle heterohybridoma cells in vivo in cows and calves were unsuccessful (Kennedy et al., 1988).

LARGE SCALE PRODUCTION

Although it is generally accepted that the establishment of stable heterohybridoma clones is more difficult than for murine×murine hybridomas (James and Bell, 1987), there is no reason to presume that the resultant lines are any less robust or adaptable to large scale production processes (Ostberg, 1986).

Almost all reported in vitro culture of mAb-secreting lines has been in static flask systems. Scale-up problems for similar lines have been more carefully studied in mouse×human systems. Here a range of different systems has been successfully applied, including roller bottles of 500 ml (Ostberg, 1986) and 200 ml capacity (Thompson et al., 1986); spinner flasks of 100 ml (Kitano et al., 1986) and 20 l (Thompson et al., 1986); and in perfusion systems using hollow

fibre modules (Andersen and Gruenberg, 1987; Takazawa et al., 1988) and 2 l jar fermentors (Kitano et al., 1986).

A mouse × sheep heterohybridoma secreting an IgG to testosterone has been successfully grown in stirred flasks (Groves et al., 1987a). A (mouse × cattle) × cattle line producing IgG1 to testosterone has been cultured in a number of systems including stirred flasks to 1 l volume (Groves and Murdin, unpublished data, 1988), and polyester foam matrix packed-bed bioreactors with a total medium volume of 150 ml (Murdin et al., 1988). These methods produced similar levels of antibody to those obtained in static flask culture. Cell densities sustainable in suspension culture ($0.5–1.0 \times 10^6$/ml) were also similar to those attained in static flask culture. Cultures of (mouse × cattle) × cattle heterohybridomas have also been sustained in large scale hollow fibre bioreactors (Endotronics junior and P) (Groves, unpublished data, 1988). Although peak cell densities comparable to other cell types (5×10^8 cells/ml) were readily achieved the process control systems were insufficiently developed to raise antibody production above 20 μg/ml (2–6 mg/day).

MONOCLONAL ANTIBODIES PRODUCED TO DATE

The first reports of heterohybridomas mostly concerned the production of monoclonal Igs of undefined specificity. There are now an increasing number of papers describing the successful production of mAb to particular antigens. The current list shown in Table 1 illustrates the scope and potential of the technique. At present, bovine mAb are in use for cattle blood-typing, and may well eventually replace the conventional polyclonal reagents which are expensive to produce and difficult to standardize (Tucker et al., 1987). A bovine mAb has provided evidence for the existence of a second MHC class I locus in cattle (Kemp et al., 1989), and others are being used in experiments directed towards the prevention and control of respiratory syncytial viral infections in cattle (Kennedy et al., 1988). Bovine mAb have also provided standards for bovine Ig isotypes (Srikumaran et al., 1987). A bovine mAb to testosterone has been administered in vivo during initial field trials to study immunoneutralisation (Morris, Rhind, Clayton and Groves, unpublished data, 1989). Ovine mAb to nematode parasites Ostertagia and Haemonchus are being used as probes to define protective antigens with a view to vaccine production (Anderson et al., 1989). An ovine mAb to progesterone (Groves et al., 1988b) is currently under development as an assay reagent.

Antibody affinities have been reported in only a few cases, presumably due to the difficulties in measuring affinities for viruses, bacteria, and erythrocyte antigens. The high affinities of bovine and ovine mAb directed against steroids reflect the better polyclonal response of these species relative to the murine system and demonstrate one advantage of non-rodent over rodent systems.

APPLICATIONS AND POTENTIAL

Although first reports of the use of heterohybridomas to produce mAb appeared at about the same time in the human and veterinary fields, progress to date has been much more rapid in the former than in the latter, no doubt dictated by urgent therapeutic needs. However, it is clear that non-rodent mAb of veterinary interest have an important rôle to play in the future in those specific cases where requirements cannot be met by rodent monoclonal antibodies.

Expansion of the work of Srikumaran and co-workers (1982, 1984, 1987) on the characterisation of bovine Ig classes and subclasses, and Yarmush and colleague's (1980, 1981) studies of rabbit Ig could be usefully extended to other species both for the basic investigation of the immune system and for the preparation of isotope standards. Heterohybridomas will provide material for the identification (Medrano and Dutrillaux, 1984; Tucker et al., 1984) and eventual manipulation of Ig genes. This will allow the engineering of antibodies to improve on specificities or effector functions. More drastic manipulation will permit the generation of chimaeric antibodies combining components from two or more species (Oi et al., 1983; Morrison, 1985; Neuberger, 1985).

The therapeutic and prophylactic prospects for human mAb have been reviewed recently (Larrick and Bourla, 1986) and all these ideas are potentially applicable to veterinary problems (Sherman and Markham, 1986). Perhaps the major limitation to development along these lines is the cost/benefit ratio. Murine mAb are already used in passive immunisation protocols (Sherman et al., 1983) and other replacements of serum therapy (e.g. control of clostridial diseases in sheep) may prove advantageous. Other therapeutic areas where mAb might provide the treatment of choice are acute toxin, or venom, elimination, direct blockading of viral entry, and specific targeting of drugs linked directly to the mAb, especially in the control of parasites. Idiotypic mAb have great potential as vaccines where standard approaches are not successful (Kennedy et al., 1986), for example if the antigen is undefined, or is particularly difficult to prepare, or if there are possible adverse effects of using an attenuated microorganism. MAb used in this way may also have benefits when immunity to a single or defined group of antigenic determinants is required.

MAb may also be utilised to modulate normal physiological processes by neutralising circulating hormones (Booman, 1988), or receptors at the site of action without producing drug residues or unwanted side effects. Anti-idiotypic mAb may also prove to be useful as surrogate hormones.

To date non-rodent mAb have been produced from a small selection of laboratory and farm species. Development of first generation techniques using mouse myelomas, in association with suitable screening assays, should permit the production of mAb from any desired species. Investigation of the immu-

nology of exotic species, or even those that have not been subject to controlled breeding for generations, may reveal important new information.

For species in which more general application of mAb is envisaged then the development of heterohybridoma fusion partners will be worthwhile because of the potential improvements in stability and predictability of the results.

Murine mAb are frequently criticised for their lack of effector actions, such as ability to fix complement, and for their poor affinity relative to polyclonal antisera from other species. Indeed, some substances are poorly immunogenic in mice or rats yet produce significant responses in other species (Tucker et al., 1987). Inter-specific mAb technology can be used to immortalise the high affinity antibodies of particular specificities from animals of defined immunological performance. The use of a non-destructive means of lymphocyte collection allows not only the development and decline of the immune response be studied, but also the repeated collection of lymphocytes from individual animals, identified as good polyclonal antisera producers, until mAb of the desired characteristics are isolated. In human mAb production there is increasing interest in primary and secondary in vitro immunization (Reading, 1982; James and Bell, 1987). It is claimed that this is a particularly useful approach for weak antigens (Boss, 1984). This is an area worth exploring on the veterinary side.

Currently, with the notable exception of human mAb, the term 'monoclonal antibodies' is generally regarded to be synonymous with products of murine origin. As the possibilities of antibodies derived from other species are acknowledged, the full potential of mAb for veterinary therapeutics and diagnostics will emerge. The application of the techniques described in this review will also permit investigation and exploitation of the immunological characteristics of a wide range of different animal species. Although mice will continue to provide indispensable research tools in mAb work, it is envisaged that interspecific fusion will increasingly be relied upon to provide reagents for practical and commercial applications.

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