Antibody-mediated enhancement of growth hormone activity: application to animal production

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ABSTRACT

The binding of monoclonal antibodies (MAbs) of predetermined specificity to growth hormone (GH) can result in significant enhancement of the biological activity of the hormone *in vivo*. The topographic region on GH associated with the enhancement phenomenon has been localized to two proximate sites on adjacent loop regions by extensive peptide synthesis (sequences: 35-53 and 134-154). The application of these observations to improving animal production and lactation has required the demonstration of (i) autoimmunization of animals with short peptide fragments of GH and (ii) increased production parameters following administration of particular antibodies. The latter aspect has now been demonstrated in a number of animal models by the passive vaccination of animals with purified anti-peptide antisera or monoclonal antibodies. Production parameters affected in such experiments include growth rate, fat metabolism, diabetogenic activity and lactation in ewes. At the present time, it has been shown that peptide sequence 134-154 will elicit up to 80% high titre, immuno-positive responders in sheep. Monitoring the growth rate and other production parameters of lambs vaccinated with sequence 134-154 indicates improved growth and total protein characteristics. These observations, in conjunction with the development of methodologies for effective autoimmunization of animals, may provide a basis for the design of peptide vaccines to improve animal production.

Keywords Growth hormone, immunisation, antibodies.

INTRODUCTION

The improvement of animal production by hormone-based means is coming under increasing pressure from the authorities and the public. Although this view may be considered rather uninformed, particularly for the use of recombinant growth hormones (bGH and pGH) which do not promote growth in primates and have very short half-lives, it is clear that the perception of 'hormone-free' meat is becoming more desirable both from the public's stand-point and by those who wish to employ the image for marketing purposes.

Immunological methods for the control of animal production and fertility have been researched for nearly 20 years, however, it is not until recently that the first autoimmunocastration vaccine has been available commercially (Hoskinson *et al.*, 1990). This product, which is essentially an antigenic formulation of the hypothalamically produced hormone LHRH (luteinizing hormone releasing hormone), causes animals to produce hormone-neutralizing antibodies with consequent inhibition or reversal of sexual maturity. Unlike hormone-based products for the management and improvement of animal production, the vaccination approach utilizes minute quantities, of often biologically inactive hormone. Indeed, the application of this approach to growth hormone, or even to the prevention of animal diseases, only requires the employment of a small portion of the antigenic repertoire of the hormone, virus or bacterium.

There are currently four immunological approaches to the improvement of GH-regulated animal production under investigation; one of these involves the induction of neutralizing anti-somatostatin antibodies with the view of increasing circulating GH levels (Spencer *et al.*, 1983; Bass *et al.*, 1987). More recently, it has been shown that the activity of GRF (growth hormone releasing factor) can be significantly increased by site-directed antisera to the peptide. The mechanism of enhancement is thought to be due to the longer half-life of the GRF-antibody complex (Pell *et al.*, 1990). The remaining two approaches are based on either

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TABLE 1 Antibody mediated enhancement.

Proposed Mechanism	Molecule/Mediator	Reference
Prolonged half-life/slow release	Insulin	Dixon *et al*, 1975
	Bradykinin	Goodfriend *et al*, 1970
	TSH	Goodfriend *et al*, 1970 Holder *et al*, 1987
	α-MSH	Goodfriend *et al*, 1970
	Angiotensin	Goodfriend *et al*, 1970
Bivalency	EGF	Shechter *et al*, 1979a
	Insulin	Shechter *et al*, 1979b
	HLA-A2	Holmes and Parham 1983
Fc-region targetting	MDP	Leclerc *et al*, 1984
Conformational	β-galactosidase	Frackelton and Rotman 1980
	MHC (class 1)	Diamond *et al*, 1984
Restriction see text	hGH	Holder *et al*, 1985 Aston *et al*, 1986
	bGH	Aston *et al*, 1987
	γ-interferon	Schreiber *et al*, 1985
	insulin	Shechter *et al*, 1979a

immunologically enhancing GH activity or mimicking this effect with anti-idiotypic vaccines (Holder *et al.*, 1991).

ENHANCEMENT OF HORMONE ACTIVITY BY ANTIBODIES

There is now a growing body of evidence to show that antibodies can enhance hormonal activity (Table 1; Aston *et al.*, 1989). The models in which such enhancement phenomena have been demonstrated vary considerably and may themselves only provide an artifactual observation in so far as *in vivo* effects are concerned. For example, most diabetic patients with circulating antibodies to insulin can manifest prolonged insulin activity following a bolus injection of the hormone. However, vaccination of a non-diabetic individual against their own endogenous insulin is likely to result in inhibition of circulating hormone activity. Similarly, antibody-mediated enhancement phenomena which are dependent on the corresponding complex having access to several different receptors (Aston *et al.*, 1989), may not manifest in *in vitro* systems where usually only one receptor type is available or indeed selected for.

The known regulatory processes affecting hormone function *in vivo* are diverse; these include factors such as hormone production, clearance, circulating binding proteins, receptor diversity and their tissue predominance and the corresponding genetic factors controlling expression of both the hormone and their receptors. The binding of antibodies to a hormone may cause effects at several different levels of the regulation cascade. Perhaps the simplest hormonal enhancement concept by antibody *in vivo* would be the retardation of systemic clearance of exogenously administered hormone (Dixon *et al.*, 1975). This approach is clearly of little value to the enhancement of endogenous hormone activity, which is often considered to be released by the endocrine gland at optimum levels. Support for this view come from the observation that optimum GH release from the pituitary is pulsatile; continuous administration of equivalent doses of GH results in less growth promotion than that observed for

hormone delivered in a pulsatile fashion (Clark *et al.*, 1985). Similarly, a single bolus injection is considerably less potent than either continuous or pulsatile administration. However, as indicated above, there are many levels at which hormonal activity can be regulated; the optimal level of hormone delivery will also depend on the levels of circulating binding protein which, in its simplest form, may act as a 'buffer' reservoir. In practice, such circulating binding proteins (receptors) bind to the hormone in a highly specific fashion, thus further restricting the binding of the resulting complex to tissue receptors of distinct specificity. These concepts are summarized schematically in Figure 1. Essentially, it is proposed that antibodies can be of either inhibitory or enhancing specificity with a further group binding to non-functional sites and consequently being devoid of either enhancing or inhibitory activity. Enhancing antibodies are proposed to 'restrict' or 're-direct' the resulting complex to receptors relevant for the growth process. In a similar way, circulating binding proteins may be characterized by their ability to direct the hormone to particular receptors or tissues, thus not solely acting as passive carriers. Although much remains unanswered in such a schematic representation, it provides a tangible framework by which to account for enhancement of endogenous GH activity by antibody.

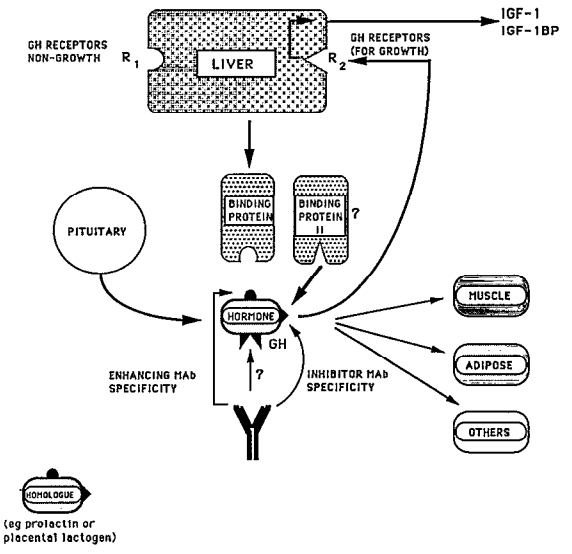

FIG 1 Schematic representation of certain aspects of the hormone action cascade. A hormone (eg. GH), has binding sites on a variety of cells/tissues with some tissues demonstrating more than one type of binding site. Circulating binding proteins (or membrane released receptors) are released into the circulation by certain tissues. Due to their nature, such binding proteins can 'restrict' the binding of the hormone to particular receptor subsets. Different tissues may have functionally distinct receptors for the hormone. Homologues (eg. prolactin/placenta lactogen) can only bind and mask certain sites on the hormone and consequently, 'restrict' the hormone to particular receptor subsets. Such a region on the hormone may be similar to that recognized by a circulating binding protein.

ENHANCEMENT OF GROWTH HORMONE ACTIVITY

Growth hormone is believed to mediate its effects by either inducing the production of insulin-like growth factor-1 (IGF-1) in liver tissue, with consequent release of the growth factor into the circulation, or by acting directly on certain tissues. At the present time it is still not clear whether all GH activity is mediated through IGF-1 or related growth factors. What is clear, however, is that GH has distinct activities on different tissues, probably mediated through structurally distinct receptors (Barnard *et al.*, 1985; Barnard and Waters, 1988; Schepper *et al.*, 1984.) The receptor diversity for GH appears to stem from variation in post-translational modification of the receptor; indeed, the serum binding protein for GH corresponds to the extracellular domain of the plasma membrane receptor (Leung *et al.*, 1987).

There is now a considerable body of evidence to show that complexing GH with particular MAbs, or with polyclonal antibodies of restricted specificity, results in significant enhancement of biological activity (Aston *et al.*, 1989; Pell *et al.*, 1990). Analysis of the nature of the enhancement phenomenon by titration of both MAb and hormone is shown in Figure 2. In the presence of a significant molar excess of MAb binding sites to hormone (two/MAb), there is saturation of the enhancement effect. No reversal of the enhancement of GH activity can demonstrated by further increasing the dose of MAb (data not presented). However, in the presence of a second MAb, binding to a topographically

distinct site, there is not only reversal of the enhancement effect but also inhibition of basal GH activity (Aston *et al.*, 1986). The reason for the resulting inhibition of GH activity following the binding of two MAbs of non-overlapping epitope specificity, probably stems from the resulting polyvalency of such complexes. Indeed, such complexes are rapidly cleared by the immune system, whereas complexes between a single MAb and GH are soluble and can, in certain cases, remain in the circulation for many hours.

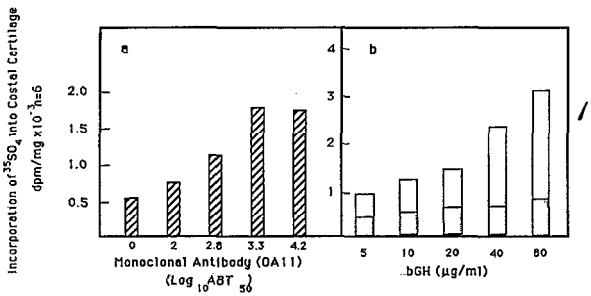

FIG 2 Enhancement of bovine growth hormone activity (bGH) byi monoclonal antobody OA11 in hypopituitary dwarf mice. Growth rates were determined by incorporation of sulphate into costal cartilage in groups of six animals (Aston *et al.*, 1987). Each animal received one injection of hormone or complex/day on two consequtive days. (a) Dose response of MAb OA11 (1 ABT50 = 10 ng Ig) in the presence of a constant amount of bGH (50 µg). (b) Dose response of bGH (5-80 µg), in the presence (open bar) or absence (shaded bar) of MAb OA11.

Antigenic mapping of the GH enhancing specificities by analysis of antisera to a variety of peptide sequences has shown that the region associated with the phenomenon is located on a long loop linking helices 3 and 4 and including the sequence region 134-154 (Figures 3 and 4). Support for this topographic location for the enhancing site on GH is derived from the fact that antisera to a sequentially distinct, but topographically proximate, region (35-53), are also enhancing (see Figure 3; Bomford and Aston, 1990). It is interesting to note that sequence 32-46, which does not lie within the indicated enhancing region (Figure 4), failed to generate enhancing antisera (Figure 3), despite its substantial overlap with peptide sequence 35-53.

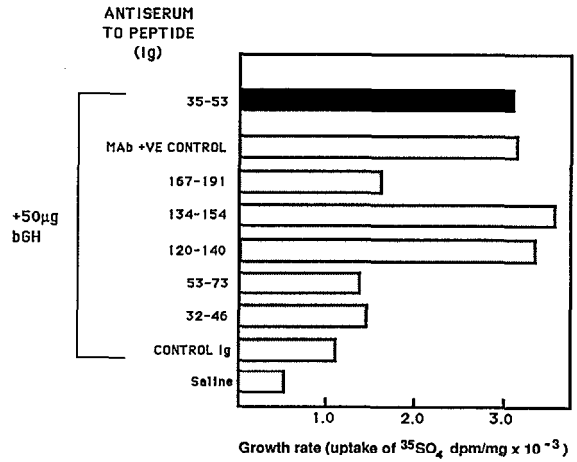

FIG 3 Enhancement of bovine GH activity in dwarf mice by site directed antisera. The growth response of hypopituitary dwarf mice to injected GH is associated with incorporation of 35-S labelled sulphate into costal cartilage (Aston *et al.*, 1987). Growth rates in response to bGH (50 µg) in the presence of control or anti-peptide antisera are shown as means of groups of 6 animals. Control Ig was derived from ovalbumin immunized sheep. Statistical significance was determined by Student's T-test, $P < 0.005$ for peptide antisera to sequences 35-53, 120-140, and 134-154 as well as for MAb 14.

ANTIGENICITY OF GH PEPTIDES AND ENHANCEMENT OF ENDOGENOUS GH ACTIVITY

In order for the concept of MAb (or anti-peptide)-mediated enhancement of GH activity to have application to the improvement of animal production there are two basic requirements: (i) that passive administration of antibody can enhance circulating GH activity (rather than exogenously administered hormone) and (ii) that auto-immunization of target species with GH peptides results in sufficient numbers of high responders to make the exercise worthwhile and cost effective. In the case of the former, there are now a number of experiments to show that passive vaccination of animals with MAb or anti-peptide antiserum results in significant enhancement of endogenous GH activity (Pell *et al.*, 1989 a,b; Pell *et al.*, 1990). Historically, the earliest of these experiments employed MAb raised to human GH but which cross-reacted equally well with GH from marmosets. This

antibody enabled the determination of the effects of an enhancing MAb on circulating GH activity in marmosets (Holder *et al.*, 1985). Animals treated with this antibody grew 25-30% faster than the group receiving control Ig (Table 2). Similar types of experiment, employing GH-enhancing murine MAb to bovine/ovine GH were subsequently undertaken in lambs. By using the diabetogenic activity of GH as a marker, the effects of GH, MAb only or combinations of antibody and exogenous hormone were determined (Figure 5).

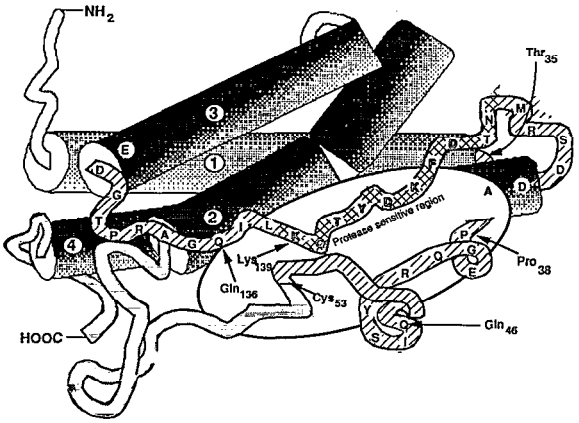

FIG 4 Schematic representation of the three dimensional structure of GH, based on Abel-Meguid *et al.* (1987). Location and amino acids sequences of peptides 120-140 and 134-154 are shown on the loop which links helices 3 and 4. A further peptide (35-53), identified previously to elicit growth enhancing antibodies (Bomford and Aston, 1989), is partially shown on the loop between helices 1 and 2. The structural relationship between the protease sensitive region (KQTYDKFDTN; dark-shaded) and the sequences that elicit growth-enhancing antibodies (diagonal lines) is shown. Cleavage at this site has been shown previously to result in enhancement in the growth promoting activity of hormone. The gray-shaded loop regions promoting activity of hormone. The gray-shaded regions, 53-73 and 167-191, represent synthetic peptides which failed to elicit growth-enhancing antibodies. The general region which may be involved in the enhancement phenomenon is indicated.

It is clear from this data that MAb OA11 can substantially enhance exogenously administered GH activity (GH+MAb), so that insulin has virtually no glucose lowering activity. However, more importantly, MAb OA11 alone induced significant effects on the diabetogenic activity of circulating GH (MAb only). A summary of the evidence to show that antibody alone can enhance the activity of endogenous GH can be found in Pell *et al.* (1990). In the latter review, we also show that treatment of lactating ewes with anti-peptide antiserum can result in enhancement of the galactopoietic action of GH. Ewes treated with an anti-GH peptide Ig for a 21 day period resulted in significant increases in milk yields; unlike the rapid decline of galactopoietic activity of GH treated animals at the end of the treatment period, the milk production of antibody treated animals remained high following the termination of treatment.

TABLE 2 Enhancement of marmoset growth by passive immunization with anti-human growth hormone monoclonal antibody (MAb-EB01). This antibody cross-reacts equally well with monkey GH and has been shown to enhance GH activity in dwarf mouse models (Holder et al, 1985). Each group (four animals) received treatment (S.C. injection) three times weekly during the experimental period. Animals receiving MAb-EB01 were injected with 0.5 mg of Ig at each administration; animals receiving control immunoglobin were treated with corresponding quantities of normal mouse Ig. Growth rates were determined by weighing prior to each treatment ($P < 0.005$ between treatment and control groups from day 30).

	Cumulative weight gain (g)			
Days after treatment	10	20	30	40
Control mouse Ig	13	28	42	58
MAb EB01	21	43	68	90

More recent studies by Pell *et al.* (submitted for publication) have shown that not only does passive administration of anti-peptide antibody improve production parameters in lambs but also active vaccination (Figure 6). In the passive aspect of this experiment lambs were treated with purified Ig from sheep immunized against peptide sequence 134-154 of GH (identical to the corresponding sequence of pGH). Such antisera cross-react with both bovine and porcine GHs with equal efficacy. Animals receiving either GH or anti-peptide antiserum had significant increases in total carcass protein; a combination of both exogenous GH and anti-peptide antiserum gave a further improvement in total protein although this was not significant over GH or anti-peptide antibody only treated groups. Within the same study, actively vaccinated animals with peptide 134-154 linked to ovalbumin (four injections of antigen in FCA), gave a significantly

improved total carcass protein over control vaccinated animals (ovalbumin only). Similarly, GH treated animals had corresponding increases in total protein; combination treatment of GH and active vaccination failed to give significant improvement over GH only or active vaccination only treated groups.

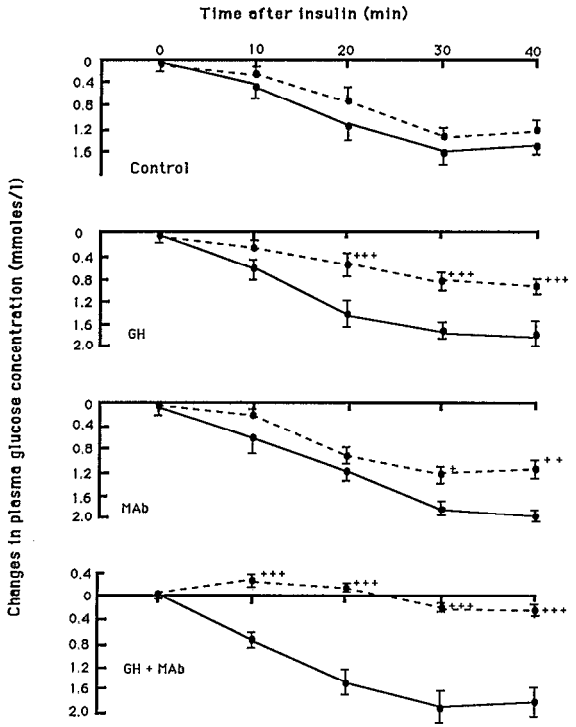

FIG 5 Enhancement of diabetogenic activity of circulating GH in lambs. Insulin tolerance tests on ewe lambs before treatment (dashed lines) with (a) phosphate-buffered saline, (b) bovine growth hormone (bGH; 0.15 mg/kg), (c) monoclonal antibody (MAb; 5.0 mg), and (d) MAb-bGH complex (0.15 mg/kg bGH + 5.0 mg OA11). The decrease in plasma glucose concentration following the administration of insulin (0.08 U/kg liveweight) is expressed relative to steady state pre-insulin concentrations. Responses before and during treatment were analysed by paired t-test for each time point (+, $P < 0.05$; ++ $P < 0.01$; +++ $P < 0.001$). Values are means ± SEM; with five animals per group.

Application of the GH enhancement approach to animal production requires the development of a GH vaccine which yields antibodies of a highly restricted specificity. Combinations of enhancing antibodies have been previously shown to result in the loss of GH enhancement (Aston *et al.*, 1986). Thus, from the immunological stand-point a peptide is required which is not only short but which can also be made to circumvent the animals tolerance to 'self' proteins. Within the highlighted topographic region shown in Figure 4 a number of peptides have been identified which can effectively generate high titre antibodies to GH when conjugated to carrier. Successful autoimmunization has generally been observed in 60-90% of animals immunized, depending on the species (Aston *et al.*, in preparation).

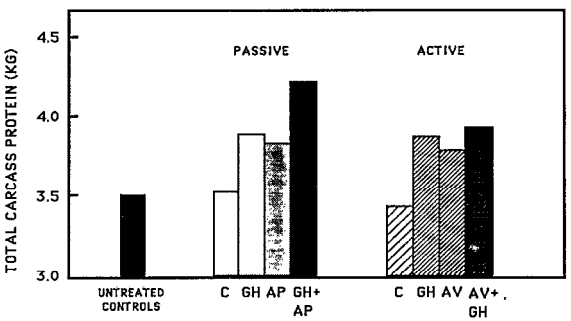

FIG 6 Enhancement of total carcass protein in lambs following passive and active vaccination against region 134-154 of GH. For the passive aspect of this experiment, 10 week old lambs were treated with: control Ig prepared from ovalbumin immunized sheep (C); growth hormone (GH 0.1 mg/kg/day); anti peptide 134-154 Ig purified from vaccinated sheep (AP) and a combination of GH (0.1 mg/kg/day) and anti peptide 134-154 Ig. Actively vaccinated animals (AV; peptide 134-154 linked to ovalbumin with gluteraldehyde) received their primary injection at 9 weeks old followed by a further 3 challenges at 2-4 weeks\ intervals. Control animals (C) received a vaccine of carrier only (ovalbumin); groups receiving GH were treated with 0.1 mg/kg/day during the 10 week treatment period, whereas the combination group (AV + GH) received the 134-154-ovalbumin vaccine as above conjunction with daily injection of hormone (0.1 mg/kg/day). All vaccines were adminstered in Freund's complete adjuvant (primary) or Freund's incomplete adjuvant (challenges).

DISCUSSION

Enhancement of hormonal activity by particular polyclonal antisera or MAbs has been documented for a number of mediators including: GH (Aston *et al.*, 1989), insulin (Shechter *et al.*, 1979a), EGF (Shechter *et al.*, 1979b), thyroid stimulating hormone (Holder *et al.*, 1987), angiotensin, bradykinin and α-MSH

(Goodfriend *et al.*, 1970), tumour necrosis factor (TNFα)(D.A. Rathjen *pers. comm.*) and γ-interferon (Schreiber *et al.*, 1985). Although a number of possible mechanisms are already known to operate during the course of antibody mediated enhancement of hormonal activity (eg. bivalency, Fc-region mediated targeting, conformational changes resulting in improved receptor interaction and slow release) none of these appear to uniquely apply to the enhancement of GH activity (see Aston *et al.*, 1989). We have proposed that there is a topographic region on GH (proximate and including the loop linking helices 3 and 4) which when associated with antibody produces a complex with highly enhanced growth promoting activity. The identification of this region was achieved following the synthesis of several hundred peptides and the generation of corresponding antisera which were subsequently evaluated in dwarf mice for enhancing activity (Aston *et al.*, in press). It is interesting to note that the above defined region partly overlaps with the proteolytically sensitive site on GH (Lewis *et al*, 1975); cleavage of GH at this site has also been proposed to produce a form of GH with enhanced activity (Singh *et al.*, 1974). We have further proposed that the binding of antibody to the enhancing site on GH results in a complex with altered receptor specificity (Aston *et al.*, 1986); such complexes may be recognizing a small group of binding sites which are uniquely relevant to the growth process. Indeed, it is known that a variety of different tissues (eg. muscle, adipose, liver and lymphocytes) have GH receptors but each tissue responds differently to a GH stimulus. Although we have not been able to provide definitive evidence that the 'restriction' hypothesis of hormonal enhancement operates for the GH model, recent studies by Rathjen *et al.* (submitted for publication) show unequivocally that a particular MAb to TNFα can permit the binding of the mediator to TNFα receptors on some tissues but not others. A consequence of this selectivity of the complex results in a 10-fold increase in the anti-tumour activity of the cytokine.

In vivo there is now evidence to suggest that the treatment of animals with certain antibodies to their own GH can result in significant changes in a number of GH-dependent production parameters. To date these include: lactation, growth, body composition, protein, water, IGF-1 and adipose (Holder *et al.*, 1988; Pell *et al.*, 1990; Pell *et al.*, submitted). At the molecular level it has also been shown that during the course of MAb-mediated enhancement of GH activity, there are significant increases in particular GH receptors and in circulating levels of IGF-1 (Wallis *et al.*, 1990;Thomas *et al.*, 1987). Further determination of the mechanisms associated with the MAb-dependent enhancement of GH activity, may provide a basis for taking this technology into other animal production applications.

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