Efficacy of plant-produced recombinant antibodies against HCG

S.Kathuria1,2,*, R.Sriraman1,2,*, R.Nath1,2, M.Sack1, R.Pal3, O.Artsaenko1,5, G.P.Talwar2, R.Fischer4 and R.Finnern4,6

1Department of Molecular Biotechnology, 52074 Aachen, Germany, 2Talwar Research Foundation, New Delhi 110068, 3Immunocrinology Laboratory, National Institute of Immunology, New Delhi 110067, India and 4Fraunhofer IME, Department of Molecular Biology, 52074 Aachen, Germany

5Present address: Experimental Hepatology, University Düsseldorf, 40225 Düsseldorf, Germany

6To whom correspondence should be addressed at: Pharmaceutical Product Development, Fraunhofer IME, Worringerweg 1, 52074 Aachen, Germany. E-mail: finnern@molbiotech.rwth-aachen.de

BACKGROUND: Antibody engineering facilitates the construction of different antibody formats [single chain variable fragment (scFv), diabody, full-size chimeric monoclonal antibody] with ease. METHODS: We constructed recombinant antibodies against HCG, which is widely used in pregnancy testing and is also produced by a number of cancers. RESULTS: The recombinant antibodies were transiently expressed in tobacco leaves to levels of up to 40 mg of pure protein per kg fresh leaf weight. Enzyme linked immunosorbent assay (ELISA) and electrophoretic mobility assay (EMSA) confirmed antibody specificity for the beta subunit of β-HCG. The efficacy was confirmed by inhibiting HCG induced testosterone production by Leydig cells in vitro and by blocking the HCG induced increase in mouse uterine weight in vivo. CONCLUSIONS: Passive immunization with recombinant HCG-specific antibodies may have clinical utility as (i) diagnostic and therapeutic tools for HCG-expressing cancers and (ii) contraceptive measures.

Key words: antibody engineering/HCG/plant expression system/recombinant antibodies

Introduction

HCG is synthesized and secreted soon after fertilization (Fishel et al., 1984) and plays a crucial role in implantation. Traditionally it has been used as an index for pregnancy (Bagshawe, 1983; Bottger et al., 1993). In addition, immunoneutralization of HCG has been proposed as a contraceptive measure (Talwar et al., 1994). Other applications of anti-HCG antibodies are in the diagnosis, prognosis and immunotherapy of cancers. Ectopic production of HCG and/or its subunits has been observed in several cancers, e.g. carcinoma of the bladder, retro-peritoneal teratoma, pancreatic carcinoma (Okamoto et al., 2001), carcinoma of the nervous system (Acevedo et al., 1997), colon (Kido et al., 1996) and cervix (Hameed et al., 1999). Malignant tumours of the bladder, pancreas, uterus and lung predominantly secrete the free beta subunit (β-HCG). β-HCG secreting tumours are reported to be more aggressive, radiotherapy resistant and have a greater propensity to metastasis (Acevedo and Hartsuch, 1996; Butler et al., 2000). Passive immunization with antibodies prevents growth of such tumours.

HCG is a heterodimeric glycoprotein of 40 kDa composed of two non-identical α- and β-subunits (Epstein and Levin, 1987). HCG and other related pituitary hormones, such as human LH (hLH), human FSH (hFSH), and human thyroid stimulating hormone (hTSH), share nearly identical α-chains. However, their β-chains show a variable degree of amino acid sequence heterogeneity, which can be specifically distinguished by monoclonal antibodies (mAbs). The mAb PIPP used for the studies presented in this publication recognizes the β-chain of HCG and does not react with other pituitary hormones (Gupta and Talwar, 1980).

HCG circulates from the trophoblast to the ovaries, and is amenable to inactivation by antibodies. Since immune response to vaccines varies greatly between individuals, passive immunization using antibodies neutralizing HCG has been proposed as a contraceptive measure.

The therapeutic use of murine mAbs is limited because they can have serious disadvantages: (i) short half-life in serum; (ii) mouse antibodies only weakly recruit the human effector functions; and (iii) the mAbs may elicit a human anti-mouse antibody response (hAMA) when administered to patients (Hasholzner et al., 1997). For in-vivo diagnostic and/or therapeutic approaches chimeric, humanized or human antibodies

*These authors contributed equally to this work
or antibody fragments are desirable in order to minimise an adverse immune response to the diagnostic and/or therapeutic agent. Small antibody fragments, such as single chain variable fragment (scFv) consist of the variable heavy and light chain antibody domains linked by a flexible peptide linker (Huston et al., 1988). These antibody fragments have been shown to penetrate tissue better and to be cleared faster from the circulation than full-size antibodies (Milenic et al., 1991), which are desirable traits in tumour imaging and therapy. However, this fragment is monovalent and due to the small size, 29 kDa, it has a fast clearance rate and this might result in a low total dose accumulation. The efficacy can be improved by using multimeric formats with increased avidity and a molecular weight slightly above the renal filtration threshold. One strategy to produce a multimeric antibody fragment is to shorten the flexible peptide linker of scFv antibody fragments to make it impossible to form monomers (Holliger et al., 1993). These so-called diabodies have a molecular weight of 60 kDa and they have been shown to be stable under in-vivo conditions (Adams et al., 1993; Nielsen et al., 2000).

Antibodies are needed in large quantities for many medical and biotechnological applications. They can be produced in heterologous expression systems such as microbes, animal cells, transgenic animals, plant tissue culture, transient plant expression and transgenic plants. While bacteria are an inexpensive, convenient production system, they are incapable of many of the post-translational modifications necessary for the activity of many mammalian proteins. These limitations, the cost of expression of proteins in mammalian cells and potential safety issues prompted us to explore plants as a cheap, safe and efficient alternative. Antibodies have already been successfully expressed in plants (Hiatt et al., 1989; Ma and Hein, 1995; Voss et al., 1995). They are functionally equivalent to those produced by hybridoma (Hiatt et al., 1989; Voss et al., 1995) and further refinements have made it possible to produce chimeric mouse–human therapeutic antibodies transiently in plants in sufficient quantities for preclinical trials (Zeitlin et al., 1998; Vaquero et al., 1999).

In order to investigate the efficacy of different antibody formats specific for HCG, we engineered the scFv (Huston et al., 1993), diabody (Hudson and Kortt, 1999) and full-size chimeric (Morrison et al., 1984) mAb PIPP and expressed all three recombinant antibodies transiently (Vaquero et al., 1999; Fischer and Emans, 2000) in tobacco leaves to levels of 20–40 mg of pure antibody per kg fresh weight of leaves. ELISA and EMSA confirmed antibody specificity to β-HCG and HCG. Antibody efficacy was confirmed in vitro by inhibiting HCG induced production of testosterone by Leydig cells and blocking HCG induced rise in mouse uterine weight in vivo. These HCG specific recombinant antibodies may have clinical utility as (i) contraceptive measures and (ii) diagnostic and therapeutic tools of HCG expressing cancers.

**Materials and methods**

**Construction of recombinant antibody fragments**

scFv and diabody PIPP

Total RNA was isolated from the hybridoma cell line producing the mAb PIPP using Trizol (Invitrogen, Eggenstein, Germany). cDNA was prepared using constant domain specific primer for the murine IgG1 heavy (5′GGCCAGTGGAAGACAGA3′) and kappa light (5′GGTG-ATGCTGACCACACTGTA TCCGCGACGGGGCGAC-TAGT3′) antibody chains using a reverse transcription-polymerase chain reaction (RT-PCR) kit (Amersham Pharmacia Biotech, Freiburg, Germany). The variable antibody domains of the heavy and light chains were amplified by PCR. The amplicons were gel purified and cloned into the phage display vector pHen4-II (Zhang et al., 2001). The scFv antibody construct contained the 218 linker (Whitlow et al., 1993)(5′GGCTCCACCTCAGGCTCCGTAAACCTGGCCCTGGG GAGGGATCAACTAAGGGCGGGCCT3′) and the diabody construct contained an eight amino acid linker (5′GGCTCCACCTCAGGCGGCGCA3′). The constructs were expressed in E. coli and screened for binding to HCG followed by subcloning of the variable antibody genes into the plant expression vector pSSH1 (Voss et al., 1995) as previously described (Vaquero et al., 1999). The constructs contained a six-histidine tag (His6) to allow purification by metal ion affinity chromatography (IMAC) (Hochuli et al., 1988).

**Chimeric full-size antibody PIPP**

Chimeric mAb PIPP heavy and light chain genes were generated by exchanging the mouse light and heavy chain constant domain sequences with their human counterparts (kappa, IgG1) using splice overhang extension (SOE)-PCR and cloned into two independent plant expression vectors as previously described (Vaquero et al., 1999). In vivo assembly of full-size chimeric mAb PIPP was achieved by co-expression of the chimeric light and heavy chains after vacuum infiltration of tobacco leaves with the respective clones of recombinant Agrobacterium. The chimeric constructs contained the endoplasmatic reticulum (ER) retention signal KDEL (Denecke et al., 1992) that facilitated targeting of the protein to the ER.

**Plant cultivation**

*Nicotiana tabacum*, cultivar Petit Havana SR1, was cultivated in the greenhouse in DE73 standard soil. Leaves from 3–6 week old plants were used for vacuum infiltration.

**Agrobacterium mediated transient expression of scFv, diabody and chimeric mAb PIPP**

Agrobacterium tumefaciens strain GV3101 were transformed by electroporation with the plant expression vectors pSSH1 containing scFv PIPP, diabody PIPP or chimeric heavy and light chain constructs of mAb PIPP. Transformed cells were incubated for 3 days at 28°C on YEB plates [0.5% (w/v) nutrient broth, 0.1% (w/v) yeast extract, 0.5% (w/v) bacitryptone, 0.5% (w/v) sucrose, 2 mmol/l MgSO4, pH 7.4, 1.5% agar] containing 100 µg/ml carbenicillin, 25 µg/ml kanamycin and 100 µg/ml rifampicin. Recombinant clones were analysed by PCR and cultured in liquid YEB media containing antibiotics for 2–3 days on a gyratory shaker at 28°C. Cells were harvested by centrifugation and resuspended in 1/20 of the initial volume of medium. Stocks were frozen at –80°C in 50% (v/v) glycerol containing 100 mmol/l MgSO4, 25 mmol/l Tris, pH 7.4. For transient expression, YEB medium containing antibiotics was inoculated with recombinant Agrobacterium and incubated overnight at 28°C. Cells were harvested and resuspended in induction medium [YEB medium, pH = 5.6, 20 µmol/l acetosyringone, 10 mmol/l 2-N-morpholino-ethanesulphonic acid (MES)] and incubated overnight at 28°C. The bacteria were centrifuged and resuspended in MMA medium [4.43 g/l Murashige and Skoog (MS) basal medium, 20 g/l sucrose, 10 mmol/l MES, pH 5.6] containing 200 µmol/l acetosyringone. The density of the suspension was adjusted to OD600 = 1.0 and the bacteria were incubated for 2 h at room temperature.

Vacuum infiltration of Agrobacterium was carried out as previously described (Kapila et al., 1996). For co-infiltration experiments equal
volumes of *Agrobacterium* cultures carrying the chimeric mAbl PIPP heavy and light chain constructs were mixed and used for vacuum infiltration.

**Extraction of recombinant protein from infiltrated leaves**

Proteins were extracted from infiltrated leaves (Vuquero et al., 1999). Briefly, 1 kg of infiltrated tobacco leaves was ground in liquid nitrogen to a fine powder. Soluble protein was extracted using 2 ml of extraction buffer (200 mmol/l Tris-HCL, 5 mmol/l EDTA, 0.1 mmol/l DTT, 0.1% Tween20, pH 7.5) per gram of leaf material. Cell debris was removed by centrifugation (20 000 g for 30 min at 4°C). The supernatant was used for purification and characterization of the recombinant proteins.

**Protein quantification**

Absorbance at 280 nm was measured with a UVIKON photometer. Absorbance was monitored by UV 260 nm and 280 nm, with Dextran Blue additionally monitored by 610 nm.

**Protein purification**

For purification via metal ion affinity chromatography (Hochuli et al., 1988) (scFv and diabody) or Protein A (chimeric mAbl) plant extracts were centrifuged at 15 000 g for 30 min at 4°C and the supernatant was used for further processing. The pH of the supernatant was adjusted to 8.0, and 500 mmol/l NaCl was added to prevent any non-specific interaction with the purification matrix. The extract was incubated at 4°C for 1–2 h and centrifuged at 7500 g for 30 min. Supernatant was filtered through Whatman paper and applied to purification matrices. Proteins were purified following manufacturers instructions.

**Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting**

Purified proteins (1–10 µg/ml) or 4 µl plant extract were separated on a 12% SDS-PAGE under reducing conditions or on an 8% SDS-PAGE under non-reducing conditions. Proteins were visualized by staining with 0.05% (w/v) Coomassie brilliant blue. For estimation of molecular weights the M12 marker (BioRad, München, Germany) was used.

For Western blot analysis proteins were electro-transferred to nitrocellulose membranes (Amersham Pharmacia Biotech, Freiburg, Germany). Membranes were blocked for 1 h at room temperature with 4% (w/v) skimmed milk dissolved in PBS. Bound scFv and diabody were detected with a mouse-anti-KDEL (StressGen Biotechnologies, York, UK) or anti-HIS\textsubscript{6} (Qiagen, Hilden, Germany) antibody followed by a goat-anti-mouse alkaline phosphatase (AP) conjugated antibody. Chimeric full-size antibody was detected by AP conjugated polyclonal goat anti-human antibodies (heavy and light chain specific) (1 µg/ml). Proteins were visualized by incubation with Nitro Blue Tetrazolium chloride/5-bromo 4-chloroindol-3-yl phosphate (NBT-BCIP, Pierce, St. Augustin, Germany). Between incubation steps membranes were washed three times with PBS containing 0.05% Tween 20.

**ELISA**

Microtiter plates (M129B; Greiner Bio-One GmbH, Solingen, Germany) were coated with 100 ng HCG (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) per well in 50 mmol/l bicarbonate buffer pH 9.6 overnight at 4°C and after washing blocked with 300 µl/well 1% (w/v) BSA in PBS for 2 h at room temperature. Plant extracts or purified proteins were serially diluted and transferred to coated and blocked plates. Bound scFv and diabody were detected with a mouse-anti-KDEL (StressGen Biotechnologies) or anti-HIS\textsubscript{6} (Qiagen) antibody followed by a goat-anti-mouse AP conjugated antibody. Chimeric full-size antibody was detected by AP conjugated polyclonal goat anti-human antibodies (heavy and light chain specific) (1 µg/ml). The ELISA was developed with 1 ng/ml p-nitrophenyl phosphate in substrate buffer [1.5% (v/v) ethanolamine, 0.15 mol/l NaCl, 1 mmol/l MgCl\textsubscript{2}, pH 9.8]. A\textsubscript{405nm} was measured using a microplate reader (Molecular Devices, Munich, Germany) and evaluated using Microcal Origin 5.0. Between all incubation steps plates were washed three times with PBS containing 0.05% Tween 20.

For competition ELISA 50 ng/ml hybridoma derived murine mAbl PIPP was added as competitor.

**Electrophoretic mobility shift assay (EMSA)**

The electrophoretic mobility of varying amounts of HCG added to 2 µg of purified scFv, diabody and chimeric mAbl PIPP was compared with the electrophoretic mobility of purified scFv, diabody and chimeric mAbl PIPP and HCG alone. Samples were loaded on a native 9% non-reducing PAGE pH 9 and run at 100V for 5 h. Proteins were visualized by Coomassie blue staining.

**Gel filtration**

A Sephadex 200 column HR10/30 (Amersham Pharmacia Biotech) was equilibrated with PBS. Affinity purified scFv, diabody and chimeric mAbl PIPP were loaded and run at 1 ml/min. Cytochrome C (12.4 kDa), carbonic anhydrase (29 kDa), BSA (66 kDa), β-amylose (200 kDa), and Dextran Blue (2000 kDa) were run as molecular weight standards (Sigma-Aldrich Chemie GmbH). The eluates were monitored by UV 260 nm and 280 nm, with Dextran Blue additionally monitored by 610 nm.

**Stability of scFv, diabody and chimeric mAbl PIPP**

Storage stability was monitored by running purified protein after storage for 6–8 months at 4°C on an SDS-PAGE and visualizing the proteins by Coomassie blue staining.

**In-vitro HCG neutralization assay of scFv, diabody and chimeric mAbl PIPP (Leydig cell bioassay)**

The Leydig cell bioassay was carried out according to an established method (Van Damme et al., 1974) as modified by Rao et al. (Rao et al., 1988). NMRI adult male mice were killed in the morning, to negate any diurnal changes in hormone levels, and the testis removed and rinsed in DMEM medium supplemented with 3% fetal calf serum (FCS). The tissues were minced and filtered through nylon membrane followed by culturing at 34°C with 5% CO\textsubscript{2} for 1 h to liberate the endogenous testosterone. The cells were pelleted for 10 min at 1000 g at 4°C and resuspended in 10 ml DMEM supplemented with 3% FCS. A total of 200 µl of cell suspension was incubated with: (i) 200 µl steroid buffer (2.35 g/l NaH\textsubscript{2}PO\textsubscript{4}, 11.2 g/l Na\textsubscript{2}HPO\textsubscript{4}, 8.8 g/l NaCl, 0.1 g/l thiomersal, 1.0 g/l gelatine); (ii) 100 µl buffer plus 100 µl HCG (concentration of HCG 250 pg/ml); or (iii) 100 µl HCG and 100 µl of recombinant antibody fragment. The reaction was mixed gently by hand and incubated for 3 h at 34°C with 5% CO\textsubscript{2}. The reaction was stopped by addition of steroid buffer and assayed for testosterone by the WHO Matched Assay Reagents as described in the WHO Methods Manual (WHO, 1993).

**In-vivo HCG neutralization assay of chimeric mAbl PIPP**

The HCG neutralization assay was carried out as previously described (Pal et al., 1990). Briefly, three groups each comprising three immature female Balb/c mice (16–18 days), were used for the assay. One group was injected with (i) 200 µl/dose saline, (ii) 200 µl/dose HCG (1.5 IU/ml) in PBS and (iii) 200 µl HCG followed by a 50 µg/ 100 µl dose of chimeric mAbl PIPP after 15 min at a different site. All injections...
Recombinant anti-HCG antibodies were i.p. The procedure was repeated on three consecutive days and on day 4 the mice were killed. The uterus of each mouse was removed and weighed.

Results

Construction and characterization of recombinant anti-HCG antibodies

The murine mAb PIPP was cloned as scFv, diabody and full-size chimeric mAb and transiently expressed in tobacco leaves (Figure 1). From 1000 g of agrobacteria infiltrated tobacco leaves 32 mg scFv, 40 mg diabody and 20 mg full-size chimeric mAb could be purified (Figure 2). Gel filtration (Figure 3) showed that the diabody and the full-size chimeric mAb PIPP eluted with the expected size of 66 kDa and 170 kDa respectively. However, the scFv eluted in two peaks, 33 kDa (expected size) and 66 kDa. A total of 80% of the scFv accumulated in the dimeric form. Free chimeric heavy chain could not be detected by non-reducing SDS-PAGE or gel filtration.

All three constructs showed binding to β-HCG and HCG in ELISA and competed with parent murine mAb PIPP (data not shown). Biological activity of the plant-expressed recombinant proteins could also be confirmed by EMSA, (Figure 4) and in gel filtration (data not shown).

Stability

Storage of the plant-expressed recombinant proteins over 6–8 months at 4°C showed the scFv to be less stable as degradation products of ~16 kDa fragments could be detected. However, the degraded scFv retained antigen-binding activity. The size of the degradation products and the retaining of the binding suggested a cleavage of the protein in the linker region. The diabody and the chimeric full-size mAb were very stable and retained nearly 100% of their binding activity (Figure 5).

Figure 1. Principle of the generation of recombinant antibodies and their production in plants. 1: Reverse transcription and PCR amplification of variable antibody genes from the RNA of a hybridoma cell line. 2: Assembly of antibody formats. scFv = single chain Fv antibody fragment consisting of the variable antibody domains of the heavy and light chain joined by a 20 amino acid linker. Diabody consisting of the variable antibody domains of the heavy and light chain joined by an eight amino acid linker. Due to the short linker this fragment cannot fold into an antigen binding site. Two of these chains have to join to form two antibody binding sites. Chimeric light chain consisting of the variable antibody domain of the light chain and the human kappa constant domain. Chimeric heavy chain consisting of the variable antibody domain of the heavy chain and the three constant domains of the human IgG constant domains. HIS6 = six-histidine residues allowing the purification of the antibody fragments via affinity chromatography KDEL = endoplasmatic reticulum (ER) retention signal allowing the accumulation of recombinant proteins in the ER. 3: Cloning of the antibody genes into a plant expression vector. 4: Transformation of Agrobacterium tumefaciens. 5: Transient expression of tobacco leaves. 6: Isolation of recombinant proteins from tobacco leaves. 7: Purification of recombinant proteins via affinity chromatography. 8: Analysis of recombinant proteins.
**In-vitro neutralization of HCG by plant-produced recombinant antibodies**

All three plant-expressed recombinant proteins were able to inhibit the stimulation of HCG production in Leydig cells (Figure 6). The scFv and diabody PIPP had the same efficacy in neutralizing HCG activity. However, the chimeric full-size mAb PIPP was nearly three logs more efficient in neutralizing HCG than the scFv or diabody.

**In-vivo neutralization of HCG by plant-produced recombinant antibodies**

Plant-produced recombinant antibodies were able to neutralize HCG in vivo (Figure 7). The saline control mice showed no change in uterine weight whereas the mice injected with HCG had a 4-fold increase in uterine weight. The mice injected with HCG and the plant-expressed chimeric antibody showed no increase in uterine weight. Their uterine weight did not differ from the control mice, confirming the efficacy of the plant-expressed chimeric mAb PIPP in vivo.

**Discussion**

Using antibody engineering techniques we generated an scFv, diabody and full-size chimeric antibody of the murine mAb...
Recombinant anti-HCG antibodies

Figure 4. Electrophoretic mobility shift assay of affinity purified recombinant proteins. Purified scFv, diabody or chimeric mAb PIPP were incubated with increasing amounts of HCG. Lane 1: 5 µg HCG + diabody; Lane 2: 2 µg HCG + diabody; Lane 3: 1 µg HCG + diabody; Lane 4: diabody; Lane 5: 5 µg HCG + scFv; Lane 6: 2 µg HCG + scFv; Lane 7: 1 µg HCG + scFv; Lane 8: scFv; Lane A: cPIPP; Lane B: HCG; and Lane C: 2.5 µg HCG cPIPP. F = free, C = complexed, arrows indicate direction of electrophoretic mobility shift.

PIPP. The recombinant antibody transiently produced in tobacco leaves retained its specificity for β-HCG and HCG. It was able to inhibit HCG-induced production of testosterone by Leydig cells in vitro and the HCG-induced rise in mouse uterine weight in vivo. The chimeric full-size mAb was 1000 times more efficient in neutralizing HCG than scFv and diabody. It was not surprising that the efficacy of scFv in neutralizing HCG in vitro was nearly identical to the diabody format as the scFv mainly forms dimeric molecules. The diabody used for these studies consisted of a fusion of heavy and light chain variable antibody domains by a short eight amino acid linker, forcing the expressed domains to attach to a complementary chain in solution to create an antigen-binding site. We speculate that the two binding sites created by this format are not as flexible in binding two HCG molecules as the chimeric full-size mAb or that higher concentrations of the dimeric format might be necessary for in-vitro and in-vivo neutralization purposes.

It was possible to purify 20–40 mg of recombinant antibodies from 1 kg of vacuum infiltrated tobacco leaves in only 3 days. In addition, we could show that co-infiltration of tobacco leaves with two populations of Agrobacteria containing either the heavy or light chain cDNA was efficient in producing functional full-size chimeric mAb. Moreover, the transient expression system is a timesaving and cost-effective alternative to other eukaryotic systems in expressing proteins, especially complex (full-size IgG) recombinant proteins. The chimeric mAb PIPP had a high affinity for binding HCG (KD = 3 × 10^{-10} mol/l). The large quantities in which this antibody can be readily produced by transient plant expression technologies provides high quality recombinant proteins suitable for diagnostic purposes such as pregnancy testing, detection of HCG synthesizing cancer cells, Downs syndrome pregnancies (Knight et al., 1998) or pre-eclampsia (Bahado-Singh et al., 1998).

Work is in progress to fully humanize this antibody (R.Sriraman, M.Sack, H.Speigel, G.P.Talwar, R.Fisher and...
R.Finnern, unpublished data) so that it can be used for detection and regulation of fertility and as a tool for the construction of improved cancer diagnostics and therapeutics. The possibility that plant-derived therapeutic proteins could elicit an immune or sensitization response in patients is of concern, although clinical application might still be possible depending on the dose requirements and blood clearance rate (Miele, 1997). In addition, glycosylation differences might not be an issue for non-glycosylated proteins and peptides, such as scFv or diabodies, and may only be a problem for therapeutic glycoproteins administered to patients by i.v. injection. Plant-derived antibodies given orally have been shown in human trials not to elicit titres of serum IgG, IgA or IgM antibodies capable of binding to the foreign protein (Ma et al., 1998). The HCG specific recombinant antibodies are currently being tested in marmosets. It might be feasible to use these plant-expressed proteins in immunoadsays and for in-vivo contraceptive measures. On radio-tagging they can also be used for imaging of HCG-synthesizing tumours and their metastasis, a purpose for which the use of a mouse mAb bears potential risks. Other useful applications for these antibodies would include the immunotherapy of cancers. Antibody fragments and chimeric full-size antibodies have already been used with benefit for immunotherapy of cancers (Baselga et al., 1996; McLaughlin et al., 1998; van Zanten-Przybysz et al., 2001). The benefits and risks are evident from such studies.

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References


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