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Human Insulin-Like Growth Factor I (IGF-I) Produced in the Mammary Glands of Transgenic Rabbits: Yield, Receptor Binding, Mitogenic Activity, and Effects on IGF-Binding Proteins

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ABSTRACT

Insulin-like growth factor I (IGF-I) has acute insulin-like metabolic effects and long-term anabolic actions offering a range of important therapeutic applications. To evaluate a system for large-scale production of this peptide in the mammary glands of transgenic livestock, we generated transgenic rabbits carrying fusion genes in which a synthetic DNA coding for human IGF-I (hIGF-I) was placed under the transcriptional control of regulatory elements isolated from the bovine α_{S1}-casein (α_{S1}-cas) gene. Western blot analysis of milk from α_{S1}-cas-hIGF-I transgenic rabbits demonstrated production of high amounts of mature hIGF-I peptide (7.6 kDa). Quantitative analysis by RIA revealed hIGF-I levels between 50 and 300 μg/ml milk. Recombinant hIGF-I purified from the milk of α_{S1}-cas-hIGF-I transgenic rabbits bound to IGF-I receptors on human IM-9 lymphoblasts and stimulated DNA synthesis by growth-arrested MG-63 human osteosarcoma cells as efficiently as hIGF-I produced in *Escherichia coli*. Ligand blot analysis of milk serum revealed the presence of 45-kDa, 30-kDa, and 23-kDa IGF-binding proteins (IGFBPs). The 30-kDa IGFBP was shown to be IGFBP-2 by immunoprecipitation using an antiserum raised against human IGFBP-2. Secretion of IGFBP-2 was markedly stimulated by hIGF-I overproduction in α_{S1}-cas-hIGF-I transgenic rabbits. The latter displayed slightly increased milk yield, but no significant changes in total protein content or overall milk protein composition, and reared their offspring without any problems or clinical signs of impaired welfare, even after multiple lactations. Our results indicate that high amounts of biologically active hIGF-I can be produced in the mammary glands of α_{S1}-cas-hIGF-I transgenic rabbits. Local production of hIGF-I in mammary tissue is associated with increased secretion of IGFBP-2, which may prevent major biological effects by high levels of hIGF-I on the mammary gland. (*Endocrinology* **138:** 307–313, 1997)

INSULIN-LIKE growth factor I (IGF-I) is a 7.6-kDa peptide with acute insulin-like metabolic effects and long-term anabolic actions (1). The therapeutic potential of IGF-I therefore includes various disorders resulting from GH deficiency or resistance (2–5), insulin-dependent diabetes mellitus (6), and noninsulin-dependent diabetes mellitus with insulin resistance (7, 8). IGF-I is used for anabolic therapies of patients with hypercatabolism after sepsis, surgery, or critical illness (9) and of cachectic patients suffering from acquired immunodeficiency syndrome (10). A positive effect of IGF-I therapy on kidney function has been shown in patients with type II diabetes mellitus (7) and in rats with experimentally induced endotoxemic acute renal failure (11). Further potential applications of IGF-I include osteoporosis (12) and cardiomyopathy (13). Treatment with IGF-I offers an exciting approach for therapy of motor neuron disorders (for review, see Ref. 14). Clinical trials have been initiated in patients with amyotrophic lateral sclerosis and are planned in patients suffering from chemotherapy-induced peripheral neuropathies (15).

Although recombinant human IGF-I (hIGF-I) can be produced in *Escherichia coli* (*E. coli*), the mammary glands of transgenic livestock could be an alternative source to produce large amounts of this peptide. We therefore generated transgenic rabbits carrying hybrid gene constructs in which a synthetic DNA coding for hIGF-I was placed under the transcriptional control of regulatory elements isolated from the bovine α_{S1}-casein (α_{S1}-cas) gene. High amounts of correctly processed hIGF-I could be purified from the milk of these rabbits to a nearly homogenous form (16). In the present study, we investigated receptor binding and biological activity of hIGF-I synthesized by the mammary glands

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of α_{S1}-cas-hIGF-I transgenic rabbits (MGTR) as compared with *E. coli*-derived hIGF-I. In addition, we studied effects of hIGF-I overproduction in the mammary gland on milk yield, overall milk protein composition and IGF-binding protein (IGFBP) levels in milk.

Materials and Methods

Generation of α_{S1}-cas-hIGF-I transgenic rabbits

Transgenic rabbits harboring fusion genes in which a synthetic hIGF-I complementary DNA was placed, under the transcriptional control of regulatory sequences from the bovine α_{S1}-cas gene, were produced by pronuclear DNA-microinjection as previously described (16). Animals investigated in this study were founder animals described in our initial report (16), as well as their F1-offspring.

Western blot analysis

Milk from α_{S1}-cas-hIGF-I transgenic rabbits and controls was defatted by centrifugation (15,000 × *g*; 4 C; 10 min). To obtain milk serum, skim milk was ultracentrifuged (100,000 × *g*; 4 C; 10 min). Milk serum was diluted 1:5 with Tris-buffered saline (TBS; pH 7.3) and subsequently mixed with an equal volume of 2 × sample buffer [5% glycerol; 10% 2-mercaptoethanol; 4% SDS; 10 mM Tris-HCl, pH 6.8], boiled (5 min), and loaded on a 5% stacking/15% separating SDS-polyacrylamide gel. Electrophoresis was performed in a Mini-Protean® II Dual Slab Cell (Bio-Rad, Munich, Germany) for 5 min at 100 V and then for 45 min at 180 V. Proteins were transferred to Hybond-C nitrocellulose (Amersham, Braunschweig, Germany) using a horizontal semidry electroblotting system (Sartorius, Göttingen, Germany). Immunodetection of hIGF-I was performed according to Amersham's ECL Western blotting protocols using a monoclonal antibody specific for hIGF-I (MCA 520; batch 177A; Serotec, Oxford, UK; dilution 1:125) and a horseradish peroxidase-labeled rabbit antimouse IgG-antibody (A 9044; Sigma, Deisenhofen, Germany; dilution 1:500).

RIA for IGF-I

IGF-I concentrations in rabbit milk were measured by a double-antibody RIA in which excess hIGF-II is added to block the interference of IGFBPs. The antiserum used (no. 878/4) has a low cross-reactivity with hIGF-II (<0.05%). The assay was validated for milk according to the recommendations of Blum & Breier (17) and Bang *et al.* (18). Briefly, milk samples were defatted by centrifugation (15 min; 5,000 × *g*; 4 C). The skim milk was then diluted and incubated in assay buffer containing the antiserum at a final dilution of 1:105,000 and 25 ng/tube of hIGF-II (Eli Lilly, Indianapolis, IN) for 30 min at room temperature. All other assay steps were identical to the method described previously (19). The minimal detectable concentration was 0.04 ng/ml. The recovery of hIGF-I added to milk samples from control rabbits was 81 ± 11% (n = 12). The intraassay and interassay coefficients of variation were 4.8% and 8.1%, respectively.

[^{125}I]IGF-I-binding assays

Specific binding of [^{125}I]IGF-I to IGF-I receptors already has been demonstrated in human IM-9 lymphoblasts (20). A linear relationship between IGF-I binding and IM-9 cell concentration was obtained over the range of 1×10^6 to 1×10^7 cells/ml. IM-9 cells were grown in continuous suspension culture in RPMI 1640 medium (Biochrom, Berlin, Germany) supplemented with 25 mM HEPES, 10% FCS (Seromed, Munich, Germany), 200 mM glutamine, 100 U/ml penicillin G and 10 μg/ml streptomycin. Medium was changed every 3 days.

In the present experiments, we incubated 5×10^6 IM-9 cells in binding buffer with 0.078 ng/ml [^{125}I]hIGF-I (Saxon, Hannover, Germany; specific activity: 256 μCi/mg) and increasing concentrations of unlabeled hIGF-I from the mammary glands of α_{S1}-cas-hIGF-I transgenic rabbits (purified as described in Ref. 16) or produced by *E. coli* (a generous gift by Dr. Th. Müller, Ciba Geigy AG, Basel, Switzerland). The binding buffer was 50 mM HEPES, 120 mM NaCl, 5 mM KCl, 1.2 mM $MgSO_4$, 10 mM $CaCl_2$, 10 mM dextrose, 15 mM CH_3COONa, 0.1% BSA, pH 7.8. All chemicals were from Merck (Darmstadt, Germany); BSA was from Behring (Marburg, Germany). Binding equilibrium was achieved after 1 h at 15 C. The specificity of [^{125}I]IGF-I binding was further demonstrated by the inhibitory action of the monoclonal antibody αIR-3 (Oncogene Science, Uniodale, NY), directed against the α-subunit of hIGF-I-receptors (21) (data not shown). Cell-bound and free intact activity were counted in an automatic γ-counter (Berthold, Munich, Germany) with 70% efficiency. The concentration of unlabeled peptide yielding a 50% inhibition of [^{125}I]IGF-I binding (IC_{50}) was determined from competition-inhibition curves. Based on the Cheng-Prusoff relationship (22), IC_{50} is inversely related to receptor affinity.

Evaluation of biological activity

Purified hIGF-I from MGTR was compared with *E. coli*-derived hIGF-I for its ability to stimulate DNA synthesis by growth-arrested, IGF-I-responsive MG-63 human osteosarcoma cells (23). MG-63 cells (CRL 1427; batch F-11035; American Type Culture Collection, Rockville, MD) were grown to confluence in MEM with Earle's BSS (Seromed), nonessential amino acids (NEAA; Seromed), and 10% heat-inactivated FCS (Biochrom). Confluent cultures were trypsinized and cells were seeded onto 24-well plates (Nunc, Wiesbaden-Biebrich, Germany) in MEM with NEAA and 0.4% FCS (5×10^4 cells/well). After 24 h, the medium was removed, the cells were washed once with Ca^{2+}- and Mg^{2+}-free PBS (37 C), and then cultured in serum-free MEM for 48 h. The quiescent cells were then washed once with PBS and 400 ml of medium MCDB104 (GIBCO, Eggenstein, Germany) containing 0.1% BSA (A-7906; Sigma), 0.1 mM dexamethasone (Sigma), 1 mg/ml transferrin (GIBCO) and various concentrations of hIGF-I. After 22 h, the cells were pulsed with [^{3}H]-thymidine (TRK 120; Amersham; 0.4 μCi/ml) in MCDB104 with 0.1% BSA for 1 h. The cells were then washed twice with ice-cold PBS and twice with ice-cold 10% trichloro acetic acid (TCA). The completely dried TCA-precipitated material was counted with a β-scintillation counter 12 h later (2 min/sample).

Ligand blot analysis of IGFBPs in milk

Milk serum was obtained as described before, fractionated by 12% SDS-PAGE under nonreducing conditions and blotted onto nitrocellulose membranes (Millipore, Ann Arbor, MI). The blots were blocked in 1% fish gelatin (Amersham) and incubated with [^{125}I]hIGF-II (10^6 cpm/blot). Binding proteins were visualized by autoradiography (X-OMAT 228 film; Eastman Kodak Company, Rochester, NY) for 96 h (24). The signals on the film were quantified by densitometry (Image Master 1D Software, Version 1.20; Pharmacia Biotech, Uppsala, Sweden). IGFBPs were further characterized by deglycosilation and by immunoprecipitation using a specific rabbit antiserum to human IGFBP-2 (kindly provided by Dr. M. Elmlinger, Universitätskinderklinik Tübingen, Tübingen, Germany). For deglycosilation of IGFBPs, 10 μl milk serum were incubated with 2 μl Endoglycosidase F/N-Glycosidase F (Boehringer Mannheim, Mannheim, Germany) for 3 h at 37 C before ligand blot analysis. For immunoprecipitation, 2 μl anti-IGFBP-2-serum were added to 10 mg washed Protein A (Pharmacia) and preincubated for 1 h at room temperature. Subsequently, 100 μl milk-serum containing 0.5 μl of a mixture of protease inhibitors (Complete, Boehringer Mannheim) were added and incubated at room temperature for 3 h. Then the samples were centrifuged and the pellets were washed twice with PBS, resuspended in nonreducing sample buffer, and boiled for 5 min. After centrifugation, the supernatant was subjected to ligand blot analysis as described above.

Determination of milk yield, total milk protein, and overall milk protein composition

Milk was obtained from lactating rabbits using a mechanical milking device specifically developed for quantitative milk extraction (25). Milk yield was determined on at least 20 days per lactation, and mean daily milk yield was calculated. Does harboring the α_{S1}-cas-hIGF-I transgene (n = 21) were compared with nontransgenic controls (n = 5) and with α_{S1}-cas-chymosin transgenic does (n = 6) (26). Total milk protein was determined using the BCA Protein Assay Reagent (Pierce, Rockford, IL). Overall milk protein composition was analyzed by SDS-PAGE under reducing conditions as described in Ref. 27. The gels were stained with Coomassie Brillant Blue G-250.

Results

Molecular form and yield of hIGF-I from MGTR

Western blot analysis of milk serum from α_{S1}-cas-hIGF-I transgenic rabbits belonging to different lines detected high amounts of hIGF-I of correct size (Fig. 1). The concentrations of hIGF-I determined by RIA in milk samples from three transgenic rabbits at different stages of lactation ranged from 50–300 μg/ml (Fig. 2), whereas IGF-I levels measured in milk from control rabbits were below 0.8 μg/ml (data not shown). Although hIGF-I levels determined in milk from an α_{S1}-cas-hIGF-I transgenic founder doe (no. 4001) were relatively constant throughout lactation, two F1 does (no. 5255, no. 5326) from a different line (4117) displayed a 2- to 4-fold increase in hIGF-I secretion from day 4 to day 21 of lactation.

Receptor binding and biological activity

To characterize the binding of hIGF-I from MGTR to IGF-I receptors, we used IM-9 lymphoblasts, a cell line in which specific IGF-I receptors have been described (20). Equilibrium competition-inhibition binding assays were performed with [^{125}I]IGF-I and increasing concentrations of hIGF-I from MGTR or from *E. coli*. As shown in Fig. 3A, maximal binding of [^{125}I]IGF-I averaged 10 ± 0.7% with nonspecific binding of less than 1%. Competition with [^{125}I]IGF-I by hIGF-I from MGTR (IC_{50} = 10 ng/ml) and from *E. coli* (IC_{50} = 12 ng/ml) was not different. Recombinant insulin was 10-fold less potent in competition than hIGF-I (data not shown).

To further investigate the biological activity of hIGF-I from MGTR, we studied its ability to stimulate [^{3}H]-thymidine incorporation by quiescent MG-63 human osteosarcoma cells. As expected from the binding studies, both hIGF-I from MGTR and from *E. coli* increased [^{3}H]-thymidine incorporation up to 2.5-fold with an EC_{50} between 1 and 4 ng/ml (Fig. 3B). A comparison of the receptor-ligand affinities with the dose-response relationships for [^{3}H]-thymidine incorporation suggested that hIGF-I from MGTR and from *E. coli* stimulate growth via high-affinity binding to IGF-I receptors.

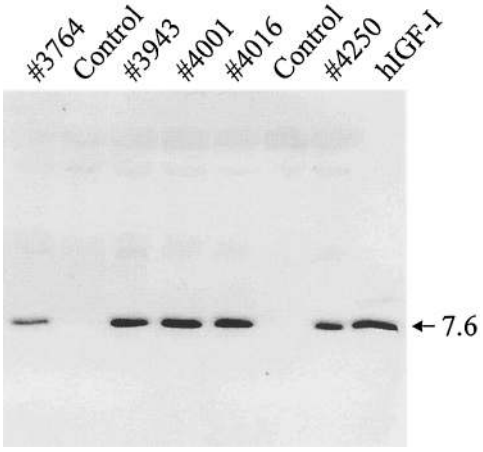

FIG. 1. Western blot analysis of milk serum samples from α_{S1}-cas-hIGF-I transgenic founder does (no. 3764, no. 3943, no. 4001, no. 4016, no. 4250) and control does. Samples were processed and immunodetection was performed using a monoclonal antibody against hIGF-I as described in *Materials and Methods*. *E. coli*-derived hIGF-I (0.5 μg) served as positive control.

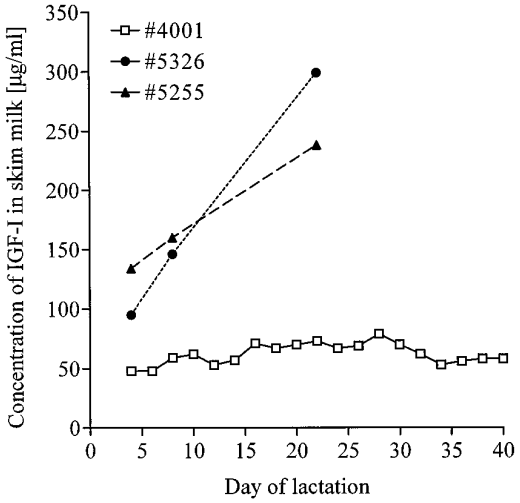

FIG. 2. Levels of hIGF-I in milk samples from a α_{S1}-cas-hIGF-I transgenic founder rabbit (no. 4001) and two F1 offspring (no. 5255, no. 5326) from a different line (4117). Concentrations of hIGF-I in skim milk were measured by RIA as described in *Materials and Methods*. IGF-I concentrations in milk samples (n = 21) from three control does ranged between 0.05 and 0.8 μg/ml (data not shown).

Effects on IGF-binding proteins

Ligand blot analysis of milk serum from α_{S1}-cas-hIGF-I transgenic rabbits and controls revealed the presence of different IGFBPs with apparent molecular mass of 45 kDa, 30 kDa, and 23 kDa. The concentration of the 30-kDa IGFBP was markedly (3- to 5-fold) increased in milk samples from transgenic rabbits, whereas the levels of the 45-kDa and 23-kDa IGFBPs were not significantly different between the two groups (Fig. 4). After deglycosilation, the molecular mass of the 45-kDa IGFBP was reduced to 39 kDa (Fig. 5A), as can be expected for IGFBP-3. In contrast, the molecular size of the 30-kDa IGFBP remained unchanged (Fig. 5A), making the presence of N-glycosilated IGFBP-4 unlikely. After immunoprecipitation of milk serum with an antiserum specific for human IGFBP-2, a single band (30 kDa) was detected in the subsequent ligand blot analysis (Fig. 5B), indicating that the IGFBP upregulated in α_{S1}-cas-hIGF-I transgenic rabbits represents IGFBP-2.

Milk yield, total protein, and overall milk protein composition

Average daily milk yield of α_{S1}-cas-hIGF-I transgenic does was slightly, but not significantly, greater than that of α_{S1}-cas-chymosin transgenic rabbits and controls (Fig. 6). When data from the latter two groups were pooled, the increase in milk yield of the hIGF-I overexpressing does reached the borderline of statistical significance (167 ± 9 *vs.* 138 ± 13 g/day; P = 0.08). Total protein content of milk from controls tended to decrease from early to midlactation and to increase again by the end of lactation. In milk from α_{S1}-cas-hIGF-I transgenic rabbits, total protein concentration was rather constant throughout lactation (Fig. 7A). The two groups were not significantly different in any stage of lactation investigated. SDS-PAGE of skim milk revealed the typical milk protein pattern described recently by Baranyi *et al.* (27) with no obvious differences between α_{S1}-cas-hIGF-I transgenic

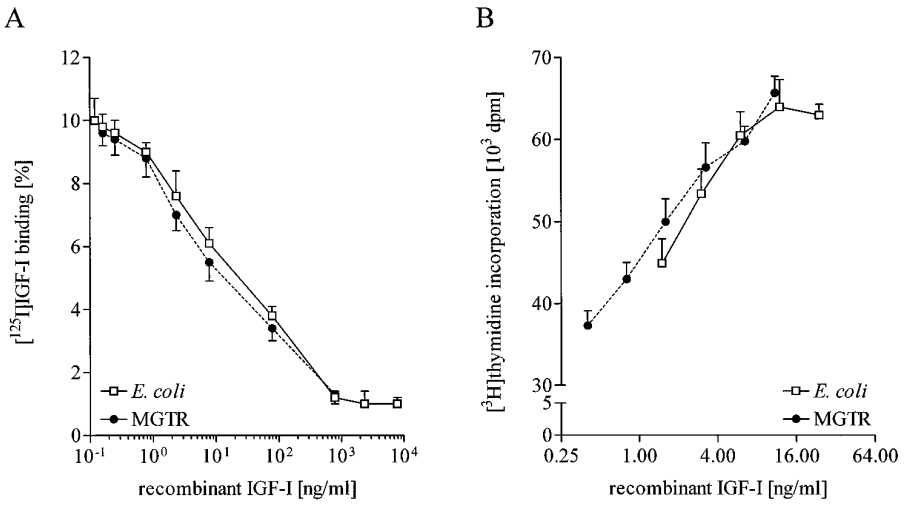

FIG. 3. Receptor binding and mitogenic activity of hIGF-I produced in MGTR as compared with *E. coli*-derived hIGF-I. A, Competition of hIGF-I (MGTR *vs. E. coli*) with [^{125}I]hIGF-I for IGF-I receptors on human IM-9 lymphoblasts. The figure shows means and SD of four determinations. B, Stimulation of [^{3}H]-thymidine incorporation by quiescent MG-63 human osteosarcoma cells. Effects by different concentrations of hIGF-I from MGTR and by *E. coli*-derived hIGF-I were determined in triplicate. The figure presents means and SD.

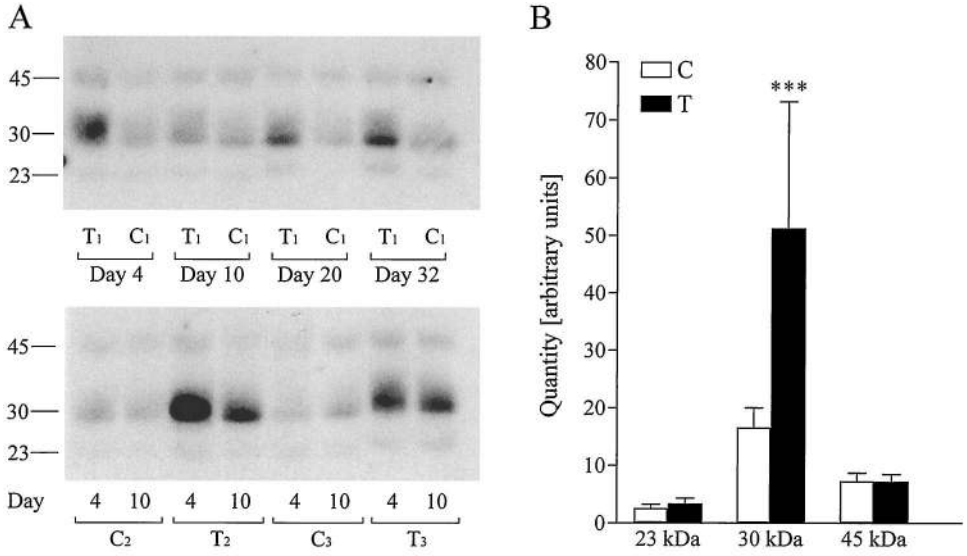

FIG. 4. Effects of hIGF-I production in the mammary glands of α_{S1}-cas-hIGF-I transgenic rabbits on IGFBP levels in milk serum. A, Ligand blot analysis of IGFBPs in milk serum samples from transgenic rabbits (n = 3; T_{1-3}) and controls (n = 3; C_{1-3}) at different stages of lactation (days 4, 10, 20, and 32 postpartum) was performed as described in *Materials and Methods* using [^{125}I]hIGF-II as tracer. B, Signals on autoradiographs were quantified by densitometry and values obtained for 23-kDa, 30-kDa, and 45-kDa IGFBPs in samples from transgenic rabbits (T), and controls (C) were compared using Wilcoxon rank-sum tests (***, $P < 0.001$). The figure shows means and SD.

rabbits and controls, except for the recombinant hIGF-I, which was clearly visible in Coomassie-stained gels (Fig. 7B).

Discussion

We have tested the technology for the production of large quantities of hIGF-I in the mammary glands of transgenic livestock by generating α_{S1}-cas-hIGF-I transgenic rabbits as a model system. Our previous study demonstrated that expression of this transgene is restricted to the mammary gland and that large amounts of correctly processed hIGF-I are secreted into milk and can be purified to a nearly homogenous form (16). The present study includes quantitative analysis of hIGF-I production throughout lactation and evaluation of its biological activity in terms of receptor binding and stimulation of DNA synthesis by growth-arrested MG-63 human osteosarcoma cells. Our results demonstrate that hIGF-I secretion is high throughout lactation, with an increase from early to midlactation. The levels of hIGF-I in milk from transgenic rabbits (50–300 μg/ml) measured by specific RIA were lower in comparison with our previous measurements by Western blot analysis (up to 1 mg/ml; Ref. 16) of the same samples. This is not surprising, given the semiquantitative nature of the Western blot methodology. However, our RIA has been shown to be free from interference by IGFBPs (17) and was rigorously validated for the rabbit milk from this study in accordance with the recommendations from the 3rd International Symposium on IGFs (18). The level of hIGF-I production in the mammary glands of transgenic rabbits was in the same order of magnitude as observed with other transgenes controlled by α_{S1}-cas regulatory sequences (26; for review, see Ref. 28).

Binding of purified hIGF-I from MGTR to IM-9 lymphoblasts was not different from that of *E. coli*-derived hIGF-I. In adherent human fibroblast monolayers, the addition of unlabeled IGF-I in concentrations between 0.5 and 10 ng/ml resulted in a paradoxical increase in [^{125}I]IGF-I binding that was caused by IGFBPs on the cell surface and their release into the medium during the binding assay (29, 30). In these experiments, unlabeled IGF-I between 25 and 300 ng/ml was required to displace [^{125}I]IGF-I binding; however, neither αIR-3 nor insulin inhibited IGF-I binding. Although IGFBPs were detected in IM-9-conditioned medium (data not

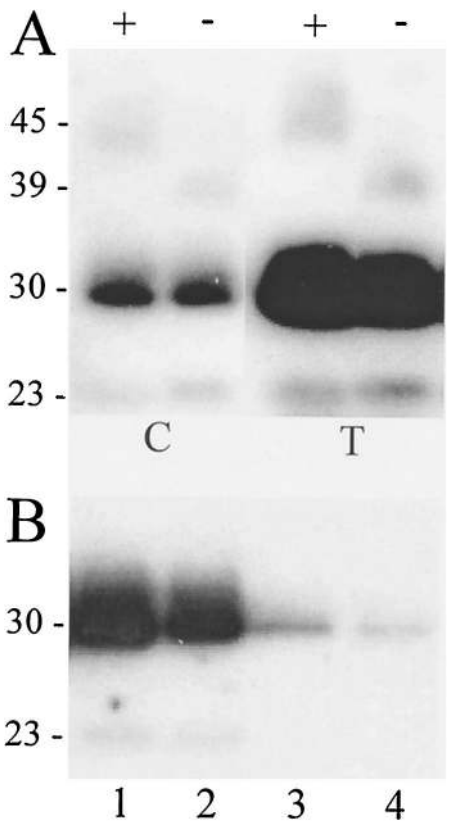

FIG. 5. Characterization of IGFBPs in milk from α_{S1}-cas-hIGF-I transgenic rabbits and controls. A, Ligand blot analysis of normal milk serum (+) and milk serum subjected to deglycosilation (−) from a control (C) and a transgenic doe (T). B, Ligand blot analysis of milk serum from transgenic rabbits (lanes 1, 2) and of material immunoprecipitated from these samples by a specific antiserum to IGFBP-2 (lanes 3, 4). Molecular mass markers are shown at the *left side* of the blots.

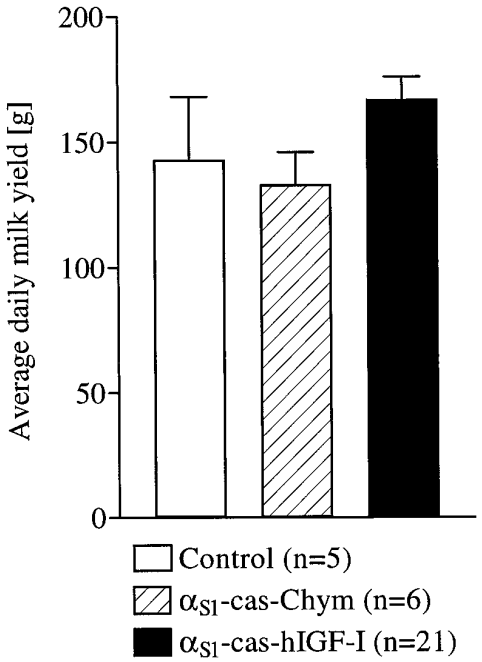

FIG. 6. Average daily milk yield of α_{S1}-cas-hIGF-I transgenic does (n = 21) as compared with rabbits (n = 6) harboring an α_{S1}-cas-chymosin (α_{S1}-cas-Chym) transgene and nontransgenic controls (n = 5). When data from the latter two groups were pooled, the increase in milk yield of the hIGF-I overexpressing does reached the borderline of statistical significance (167 ± 9 *vs.* 138 ± 13 g/day; $P = 0.08$). The figure shows means and SE.

shown), we did not observe any paradoxical increase in [^{125}I]IGF-I binding. [^{125}I]IGF-I was displaced from IM-9 cells in a dose-dependent manner by hIGF-I, by the specific type I receptor monoclonal antibody αIR-3, and with a 10-fold lower potency also by insulin. Therefore, we suppose that our IM-9 cell assay is suitable to determine IGF-I binding to type I IGF receptors without significant interference by membrane-associated IGFBPs. In addition to receptor binding, the biological activity of hIGF-I from MTGR was shown by its mitogenic effect on MG-63 cells.

In spite of the high levels of bioactive hIGF-I in the mammary glands of transgenic rabbits, these animals did not show clinical symptoms of altered function or pathology of the mammary gland (*e.g.* no difficulties in rearing their offspring), even after multiple (up to five) lactations. There was only little increase in milk yield of α_{S1}-cas-hIGF-I transgenic rabbits as compared with controls or transgenic rabbits expressing α_{S1}-cas-chymosin fusion genes.

The lack of major effects by high levels of locally synthesized hIGF-I on the mammary glands of α_{S1}-cas-hIGF-I transgenic rabbits contrasts findings of a recent study on transgenic mice in which expression of hIGF-I or des(1–3) hIGF-I was directed to the mammary gland using regulatory sequences from the rat whey acidic protein (rWAP) gene (31). Overproduction of des(1–3) hIGF-I, but not of hIGF-I, was reported to cause abnormal mammary gland development with progressive changes, including ductile hypertrophy and disorganization of secretory lobules. Mice suffering from this pathology had difficulties in rearing their pups after three to four lactations.

There are several possible explanations for the different effects observed in α_{S1}-cas-hIGF-I transgenic rabbits and rWAP-des(1–3) hIGF-I transgenic mice. First, our transgenic rabbits produce hIGF-I and not the aminoterminally shortened form des(1–3) hIGF-I that has reduced affinity for IGFBPs (32) and therefore higher biological activity (33). The markedly increased activity of IGFBP-2 in milk from α_{S1}-cas-hIGF-I transgenic rabbits could result from a feedback response of the mammary epithelial cells to locally produced hIGF-I, preventing major effects of increased IGF-I levels on the mammary glands of these animals. Stimulation of IGFBP-2 and a small complex of IGFBP-3 has been reported previously in human plasma by IGF-I administration (34). We speculate that this increase in IGFBP-2 may, at least in part, inhibit the local effects of hIGF-I on the mammary gland. Inhibition of growth of human breast cancer cells has been demonstrated for IGFBP-1 and IGFBP-3 (35, 36). On the other hand, overexpression of des(1–3) hIGF-I in transgenic mice caused altered involution and pathology of the mammary gland only on a specific genetic background (ICR × B6D2F1), suggesting that cooperation of the transgene with a specific allele in this background is necessary to result in a specific pathological phenotype (31). Hadsell *et al.* (31) did not report pathology of the mammary glands from trans-

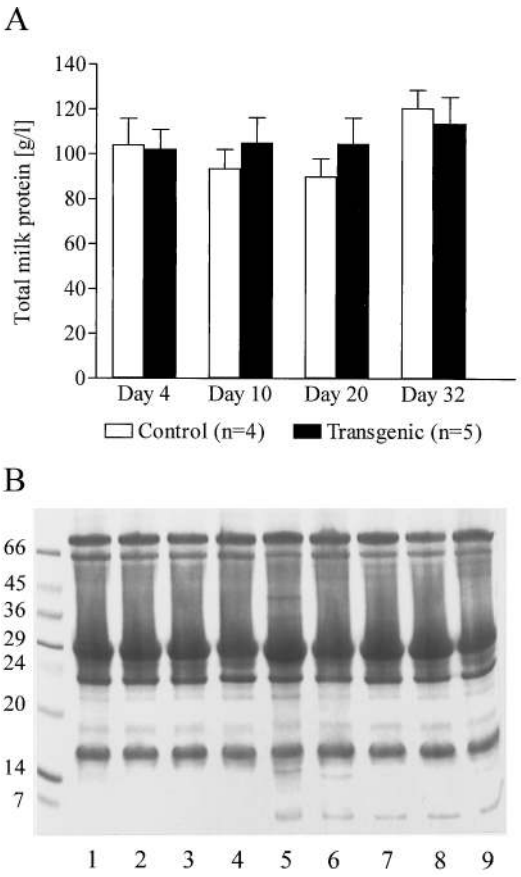

FIG. 7. Total protein concentration (A) and overall protein composition (B) of milk samples from α_{S1}-cas-hIGF-I transgenic rabbits and controls. A, The figure shows means and SD for different stages of lactation. Differences between groups within day and between days within group were tested using Wilcoxon rank-sum tests and Wilcoxon matched pairs signed-rank tests, respectively and were not significant. B, SDS-PAGE of skim milk samples (day 10 of lactation; 0.5 μl/lane) from controls (lanes 1–4) and transgenic rabbits (lanes 5–9) was carried out under reducing conditions as described in Ref. 27 and did not reveal obvious differences between the two groups, except for the recombinant hIGF-I, which was clearly visible in the Coomassie-stained gel. Molecular mass markers are in the *left* lane.

genic lines (n = 3) harboring the rWAP-hIGF-I transgene; however, immunoreactive IGF-I levels in milk samples from these animals (0.2–0.8 μg/ml) were two orders of magnitude lower than hIGF-I concentrations in milk from our transgenic rabbits. Increased activity (6-fold above control) of a 32-kDa IGFBP was reported only for one of the rWAP-hIGF-I transgenic mouse lines, whereas levels of this IGFBP were increased, on average, by 20-fold in a line carrying the rWAP-des(1–3) hIGF-I transgene. Taken together with the data from our study, these results suggest that des(1–3) hIGF-I may be more potent in stimulating an IGFBP in the range of 30 kDa than authentic hIGF-I. IGFBPs have been detected in milk from several other species, including human (19), pig (37), cow (38), and rat (39); however, to our knowledge, the present study is the first to describe IGFBPs in milk from rabbits.

In summary, our data show that high amounts of biologically active hIGF-I can be produced in the mammary glands of α_{S1}-cas-hIGF-I transgenic rabbits without compromising the animals welfare. High local production of hIGF-I markedly stimulates the secretion of IGFBP-2, which may (at least in part) inhibit the action of hIGF-I on the mammary gland. Transgenic rabbits with mammary gland-specific overproduction of hIGF-I are an important model for studying effects of this peptide on the mammary gland, thereby complementing recently published observations of rWAP-des(1–3) hIGF-I or -hIGF-I transgenic mice, with all advantages of having a second species model available.

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