The Soluble Type 2 Insulin-like Growth Factor (IGF-II) Receptor Reduces Organ Size by IGF-II-mediated and IGF-II-independent Mechanisms*

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The soluble type 2 insulin-like growth factor (IGF) receptor or IGF-II/mannose 6-phosphate receptor (sIGF2R) is produced in vivo by proteolytic deletion of the transmembrane and intracellular domains of the cellular form of the receptor (IGF2R). There is evidence that sIGF2R is a negative regulator of growth. We have shown that transgenic mice expressing an Igf2r cDNA with a deleted transmembrane domain sequence (sΔIGF2R) show reduced local organ size. In the present study, we investigate whether sΔIGF2R can slow the growth induced by an excess of IGF-II and whether the biological activity of sαIGF2R is due solely to its interactions with IGF-II. To this end, we crossed sΔIGF2R transgenics by mice overexpressing IGF-II (Blast line) or by mice carrying a disrupted paternal (active) allele of the Igf2 gene (Igf2m/m−/+). Analysis of the phenotypes revealed that the soluble IGF2R affects the size of some organs (colon and cecum) exclusively by reducing the biological activity of IGF-II, whereas in other organs (stomach and skin) the biological activity of the receptor is at least in part independent of IGF-II and must involve an interaction with other factor(s).

The growth and survival factor insulin-like growth factor II (IGF-II)1 binds to at least three different receptors: the type 1 and 2 IGF receptors and IGF2R (also known as the mannose 6-phosphate/IGF-II receptor) and the insulin receptor. Type 1 IGF receptor and the insulin receptor are members of the tyrosine kinase receptors family and mediate most of the biological effects of IGF-II (1, 2).

Genetic evidence suggests that the IGF2R gene encodes a negative regulator of growth. Many human tumors show loss of heterozygosity at the IGF2R locus frequently accompanied by mutations in the remaining allele (3–5). Furthermore, mice in which the Igf2r gene is disrupted are born 25–35% bigger than controls (6–8). IGF2R is a multifunctional protein that participates in the activation of TGF-β1, regulates lysosomal enzymes trafficking, and binds a number of ligands including proliferin, herpes simplex virus glycoprotein D, thyroglobulin, and retinoic acid (9–14).

A soluble form of IGF2R (sIGF2R) is produced by proteolytic cleavage of the transmembrane and intracellular domains of the membrane form of the receptor and is present in the serum, amniotic fluid, and urine of rodents and humans. sIGF2R binds IGF-II with high affinity in vivo and can bind mannose 6-phosphate in vitro, suggesting that it shares at least some of its ligand specificity with the membrane IGF2R (15–19). There is evidence that sIGF2R is a biologically active molecule. First, sIGF2R can inhibit DNA synthesis induced by IGF-II and epidermal growth factor in cultured rat hepatocytes (20). Second, we have obtained transgenic mice expressing a soluble IGF2R by deletion of the transmembrane domain sequence (sΔIGF2R) and fused to the regulatory sequence of the keratin 10 promoter to target expression to the alimentary canal, skin, and uterus (K10sΔIGF2R transgene). Two lines of K10sΔIGF2R transgenic mice (Kipps and Krishna) showed a 9–20% reduction of wet weight, dry weight, and water content in the alimentary canal. The effects of sαIGF2R expression were mainly local, because the organs negative for transgene expression were only marginally affected (21).

The interpretation of the biological activity of sIGF2R is complicated by the heterogeneity of its ligands. To understand to what extent the biological activity of sαIGF2R is due to interaction with IGF-II, we crossed K10sΔIGF2R transgenics by mice overexpressing IGF-II (Blast line; Ref. 22). If sΔIGF2R acts by reducing the activity of IGF-II, double transgenics Blast and K10sΔIGF2R should show an attenuation of organomegaly compared with Blast. 2) mice in which the paternal (active) allele of the Igf2 gene is disrupted (Igf2−/−). Igf2−/− mice show a growth deficiency phenotype and are fertile (23). If sΔIGF2R acts exclusively by interacting with IGF-II, organ size should not be affected in K10sΔIGF2R transgenics that are also Igf2−/− compared with Igf2−/− mice. The results of the present work provide insights into the mechanism of organ size reduction by the soluble IGF2R.

EXPERIMENTAL PROCEDURES

Transgenic Mice—K10sΔIGF2R (lines Kipps and Krishna) transgenic mice express a mutant mouse Igf2r cDNA in which the sequence encoding the transmembrane domain has been deleted to encode a soluble polypeptide (sΔIGF2R). The mutant cDNA is under the transcriptional control of the keratin 10 promoter (K10). Kipps is the line expressing the K10sΔIGF2R transgene at highest levels, and Krishna is the second best expressing line; their phenotype has been described in detail (21). K10lgf2r2/Blast and Igf2−/− mice have been described elsewhere (22, 23). All mice used in this study were heterozygotes (indicated as K10sΔIGF2R+/−, K10lgf2r2/+ and Igf2−/−) in a mixed genetic background as the integrated transgenes were bred from a F1 (C57Bl/6 × CBA) onto a 129J/Sv genetic background. In both crosses the K10sΔIGF2R transgene was transmitted maternally, because Blast fe-
males are usually not fertile, and the disrupted copy of the imprinted Igf2 gene has to be transmitted paternally (22, 23). There is no evidence that the phenotype of K10sIgf2r/+ mice is altered by the sex of the parent, which transmits the transgene.

Genotyping was performed by PCR using genomic DNA extracted from blood by using QiaAmp Mini Kit (Qiagen) or from tail tissue (24). The following protocols were used. 1) K10sIgf2r' (Kipps and Krishna lines): forward primer, 5'-ACGAGACCTGGATCAGCAGGTACC-3', and reverse primer, 5'-AGCCATCTGTCATCATCATCTGTC-3'. These two primers amplify a unique 357-bp fragment. The K10sIgf2r transgene produced a unique 256-bp fragment. The conditions were 94 °C for 1 min and 70 °C for 4 min for 35 cycles. 2) K10Igf2 (Blast line): forward primer, 5'-TGCAAGACCGCCGCTGAGGACAGC-3' (corresponding to the 3' end of exon 4 in the Igf2 gene). The conditions were 94 °C for 3 min, 53 °C for 2 min, and 72 °C for 2 min for 35 cycles. 3) Igf2mneo': forward primer, 5'-GTGTTGCTTCGTCAGCGCA-3', and reverse primer, 5'-GTGTTGCTTCGTCAGCGCAACAG-3' (corresponding to the 3' end of the neo gene). The conditions were 94 °C for 1 min and 70 °C for 4 min for 35 cycles. The neo gene produced a unique 555-bp fragment.

Analysis of Organ Parameters—Analysis of organ weights was performed on mice produced by crossing heterozygotes K10sIgf2r/+ (Kipps or Krishna line) by K10Igf2/+ or Igf2mneo' mice. Live weight was recorded, and then anesthetized mice were bled by decapitation and dissected, and the total wet weight of organs was measured after removal of fat and mesenteries. The contents of the alimentary canal were removed by gentle scraping in phosphate-buff ered saline followed by blotting in tissue paper. Organs were always dissected in the same order to normalize wet weight loss due to evaporation. Each organ was cut in two or three parts, and the parts were weighed. This made it possible to calculate the total organ dry weight, water content, DNA, and detergent-soluble protein content after assaying different parts.

For dry weight measurement, tissue fragments were dried at 65 °C for 6 days. Detergent-soluble protein was measured by the Coomassie Blue method (Bradford reagent, Sigma) after tissue homogenization in 20 mM Tris, pH 7.5, 10 mM EDTA, 0.1% Tween 20, 0.15 M NaCl. DNA content was measured in the same extract using the Hoechst 33258 fluorochrome, following addition of NaCl to 2 M final concentration and briefsonication (25). For all measured parameters, relative values (e.g. per mg wet weight) were first obtained by dividing the value obtained for a given tissue part by the weight of that tissue part. This relative value was then multiplied by the total organ wet weight to obtain the relative parameters.

RESULTS

Crosses K10sIgf2r/+ x K10Igf2/+ — To find out whether the soluble receptor could reduce growth stimulated by excess local IGF-II, two transgenes were crossed into the same mice. The transcription of both transgenes was driven by the same keratin 10 promoter, and their patterns of organ expression overlapped. The matings to produce these double transgenics also generated mice with one or the other of the transgenes. The phenotype of the single K10Igf2 r/+ transgenic is first described to provide the base line for comparison with the double transgenic.

Heterozygous transgenic mice expressing the Igf2 gene under the transcriptional control of the keratin 10 promoter (Blast line, K10Igf2r/+ ) showed local organ overgrowth as described previously (22). Organ wet weight was significantly increased in the alimentary canal, skin, and uterus compared with controls, whereas wet weight was normal in organs negative for transgene expression (column A versus column B in Tables I and II). Total dry weight followed a very similar pattern in the organs examined (column A versus column B, Tables I and II). An increase of water content relative to dry matter content (edema) has been shown in mice with elevated levels of IGF-II (8). The water content relative to dry weight was unchanged in Blast in all organs examined (columns A versus column B, Tables I and II). DNA and detergent-soluble protein contents did not change significantly in any of the organs examined, with the exception of the colon as reported (22). In this organ the total DNA content was increased by a similar extent as wet and dry weights (Table I, column A versus column B).

The phenotype of K10sIgf2r/+ mice was a reduced wet weight of the alimentary canal (21). The aim of the cross K10sIgf2r/+ x Blast was to compare the extent of organomegaly in double transgenics with wild type and Blast/+ . For these two reasons, the phenotype of K10sIgf2r/+ mice will be described in the context of the cross K10sIgf2r/+ x Igf2mneo' (see the next section; column C in Tables III and IV).

In the double transgenics, the extra soluble receptor transgene (K10sIgf2r, either Kipps or Krishna) was in the same mouse as the transgene that expressed excess IGF-II (K10Igf2, Blast line). A reduction of organomegaly was observed in most of the organs coexpressing the two transgenes. Organ wet weight was significantly reduced in double transgenics compared with Blast in the alimentary canal (column C versus column B, Table I). This reduction ranged from more than 100% in the stomach to about 50% in the cecum and colon and was more pronounced in the Blast/Kipps double transgenics (column C, Table I; Fig. 1). This result is consistent with the relative levels of the K10sIgf2r transgene expression in the two lines (21). In the skin, a significant reduction of organomegaly (~50%) was observed only in Blast/Kipps double transgenics (column C versus column B, Table I). No effect of sIgF2R expression was observed on the wet weight of the uterus (column C versus column B, Table I).

Coexpression of the two transgenes produced a decrease in organ dry weight comparable with the decrease in wet weight and did not alter DNA content or detergent-soluble protein content (column B versus column C, Table I). The decrease in dry weight was more marked in Blast/Kipps than in Blast/ Krishna double transgenics (column C, Table I). DNA content was significantly reduced in the small intestine and colon in Blast/Krishna mice compared with Blast (column C versus B). No major change in the organ parameters measured was observed in the organs, which did not express the transgene (column C versus column B, Table II).

Crosses K10sIgf2r/+ x Igf2mneo'—Mice carrying a disrupted IGF2 paternal allele (Igf2mneo') were 30% smaller than wild type at 90 days in a C57Bl/6J/CBA/129J/Sv mixed genetic background and were fertile as reported (Ref. 23; column B
**Numerical Data**

### Table I

|                | A                              | B                              | C                              |
|----------------|---------------------------------|---------------------------------|---------------------------------|
|                | **Wild type**                   | **K10igf2p/+**                  | **K10igf2p/+ K10igf2r+/+**      |
|                | (Blas)                          | (Blast)                         | Kips                           |
|                |                                 |                                 | Krishna                        |
| Body weighta   | 25.6 ± 0.7 (13)                 | 28.3 ± 0.8** (13)               | 26.2 ± 0.5 (11)                 |
|                | Stomach                         |                                 | (9)                            | 31.1 ± 1.4 (9)                 |
| Wet weight     | 158.9 ± 5.6 (13)                | 185.4 ± 5.7** (12)              | 164.5 ± 5.2**/12**              |
|                | Dry weight                      | 32.9 ± 1.3 (12)                 | 32.4 ± 1.6** (12)               |
|                | Water contentb                  | 3.7 ± 0.2 (12)                 | 3.6 ± 0.2 (12)                  |
|                | DNA                             | 0.24 ± 0.05 (10)               | 0.20 ± 0.06 (7)                |
|                | Proteinc                        | 4.3 ± 1.7 (11)                 | 5.3 ± 1.4 (7)                  |
| Small intestine|                                 |                                 |                                 |
| Wet weight     | 964.9 ± 50.7 (13)               | 1141.4 ± 27.5** (9)            | 982.1 ± 48.5** (11)            |
|                | Dry weight                      | 207.1 ± 10.0 (12)              | 194.7 ± 8.1** (12)             |
|                | Water contentb                  | 3.7 ± 0.3 (12)                 | 3.9 ± 0.2 (11)                 |
|                | DNA                             | 3.2 ± 0.7 (10)                 | 2.6 ± 0.9 (7)                  |
|                | Proteinc                        | 29.2 ± 3.9 (11)                | 32.0 ± 6.2 (7)                 |
| Cecum          |                                 |                                 |                                 |
| Wet weight     | 158.7 ± 6.3 (14)                | 282.4 ± 20.6**/10**            | 202.0 ± 9.1***/B** (12)        |
| Colun          |                                 |                                 | 205.0 ± 5.1***/B** (6)         |
| Wet weight     | 447.3 ± 24.2 (14)               | 969.1 ± 59.9** (10)            | 641.1 ± 46.9***/B** (12)       |
|                | Dry weight                      | 168.1 ± 7.5** (11)             | 121.7 ± 3.4***/B** (12)        |
|                | Water contentb                  | 4.3 ± 0.1 (11)                 | 4.3 ± 0.4 (12)                 |
|                | DNA                             | 0.9 ± 0.2 (10)                 | 1.4 ± 0.6 (7)                  |
|                | Proteinc                        | 21.4 ± 3.7 (11)                | 31.7 ± 8.9 (7)                 |
| Skin           |                                 |                                 |                                 |
| Wet weighta    | 2.7 ± 0.21 (13)                 | 4.3 ± 0.36** (9)               | 3.8 ± 0.28***/B** (11)         |
| Uterus         |                                 |                                 | 3.2 ± 0.23** (5)               |
|                | Wet weight                      | 91.2 ± 10.2 (6)                | 433.3 ± 24.1** (6)             |
|                |                                 | 404.6 ± 95.2** (6)             | 502.4 ± 59.8** (5)             |

*a* Weight expressed in grams.

*b* Water content/unit dry weight.

*c* Detergent-soluble protein.

### Table II

|                | A                              | B                              | C                              |
|----------------|---------------------------------|---------------------------------|---------------------------------|
|                | **Wild type**                   | **K10igf2p/+**                  | **K10igf2p/+ K10igf2r+/+**      |
|                | (Blas)                          | (Blast)                         | Kips                           |
|                |                                 |                                 | Krishna                        |
| Liver          | 1277.2 ± 77.7 (12)              | 1295.8 ± 64.7 (12)              | 1146.2 ± 62.5 (8)              |
| Kidneys        | 334.1 ± 14.0 (13)               | 353.4 ± 13.2 (13)               | 334.5 ± 12.2 (11)              |
| Wet weight     | 79.8 ± 3.9 (12)                 | 89.7 ± 3.1 (11)                 | 85.0 ± 3.0 (11)                |
|                | Water contenta                  | 3.1 ± 0.04 (12)                | 3.1 ± 0.03 (8)                 |
|                | DNA                             | 1.92 ± 0.45 (10)               | 2.19 ± 0.52 (10)               |
|                | Proteinb                        | 24.0 ± 5.6 (11)                | 22.4 ± 2.5 (11)                |
| Heart          |                                 |                                 |                                 |
| Wet weight     | 116.3 ± 4.6 (12)                | 122.0 ± 4.1 (12)               | 117.0 ± 2.5 (10)               |
|                | Dry weight                      | 28.2 ± 3.1 (12)                | 27.9 ± 0.6 (9)                 |
|                | Water contenta                  | 3.1 ± 0.04 (12)                | 3.2 ± 0.04 (8)                 |
|                | DNA                             | 0.38 ± 0.03 (10)               | 0.41 ± 0.05 (10)               |
|                | Proteinb                        | 6.1 ± 1.9 (11)                 | 4.8 ± 0.0 (11)                 |
| Fat            |                                 |                                 |                                 |
| Wet weight     | 107.3 ± 12.7 (13)               | 103.4 ± 16.2 (12)              | 109.1 ± 13.4 (11)              |

*a* Water content/unit dry weight.

*b* Detergent-soluble protein.

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versus column A, Table III). The levels of IGF-II transcript and peptide in Igf2p+/p− mice were below the detection limit in three independent assays. First, the IGF-II transcript was detectable in the stomach at 10 days by reverse transcription-PCR (results not shown). Second, serum obtained from Igf2p+/p− mice was included in a IGF-II radioimmunounassay as a negative control during the characterization of the K10igf2r+/+ transgenics (21). IGF-II peptide levels were below the sensitivity of the assay. Third, in an independent study in our laboratory, the stomach and small intestine of Igf2p+/p− mice were shown to contain no detectable Igf2 transcript when analyzed by a specific RNA in situ hybridization assay. Analysis of individual organs revealed that the wet weight of most organs was proportionate to the whole body weight (values of r close to 1, column B in Tables III and IV). The stomach and the skin were exceptions, because their wet weights were disproportionately larger in relation to the whole body weight (values of r larger than 1, column B in Table III). Total organ dry weights followed a pattern similar to wet weights in the

2 B. Hassan, unpublished observations.
Reduction of Organ Size by a Soluble IGF2R

Table III
Crosses Igf2m+/− × K10sIgf2r+/+; body weight and parameters of organs expressing the K10sIgf2r transgene

Values are mean weights (mg) ± S.E. in wild type (column A), mice carrying a disrupted paternal Igf2 allele (Igf2m+/−, column B), heterozygotes carrying a K10sIgf2r transgene (K10sIgf2r+/+, Kipps line, column C), and mice with both genetic modifications (Kipps and Krishna lines, column D). Figures in parentheses are the number of samples. Each value is followed by the level of significance of comparisons with wild type (w), Igf2m+/− (I) and K10sIgf2r+/− (K). Comparisons were by paired t test of sex, litter, and age matched mice. For a given organ, r is the ratio between the percentage of variation in body weight and the percentage of variation in organ wet weight, both compared with wild type. Values of r > 1 indicate that organ size is greater than predicted from body weight. *, p < 0.05; **, p < 0.01; ***, p < 0.001; no symbol, not significant.

| A | B | C | D |
|---|---|---|---|
| | Wild type | Igf2m+/− | K10sIgf2r+/+ (Kipps) | K10sIgf2r+/+/*Igf2m+/− (Kipps) |
| | | | | |
| Body weight | 26.1 ± 0.9 (13) | 17.7 ± 0.7*** (13) | 24.5 ± 1.8*** (6) | 17.7 ± 1.0*** (7) |
| Stomach | 165.1 ± 5.4 (14) | 140.2 ± 3.6*** (14) | 146.0 ± 6.9*** (6) | 93.9 ± 2.4****/+*/−/+*/− (7) |
| Dry weight | 35.9 ± 1.3 (13) | 29.8 ± 0.8*** (12) | 34.8 ± 0.9*** (7) | 22.1 ± 0.9*** (7) |
| Water content | 3.8 ± 0.2 (13) | 3.7 ± 0.1 (11) | 3.5 ± 0.3 (3) | 4.4 ± 0.4 (6) |
| DNA | 0.35 ± 0.04 (12) | 0.36 ± 0.07 (12) | 0.36 ± 0.07 (2) | 0.36 ± 0.08 (6) |
| Protein | 5.3 ± 0.7 (11) | 5.6 ± 0.59 (11) | 5.1 ± 1.0 (3) | 5.0 ± 2.1 (6) |
| Cecum | Wet weight | 140.6 ± 5.1 (12) | 178.2 ± 3.8*** (12) | 168.4 ± 8.2*** (6) | 89.5 ± 4.1*** (6) |
| | | | | | 90.4 ± 4.9*** (6) |
| Colon | Wet weight | 377.8 ± 22.8 (14) | 278.6 ± 20.6*** (14) | 302.8 ± 30.0*** (6) | 250.1 ± 19.8*** (3) |
| | | | | | 253.5 ± 22.9*** (6) |
| | Dry weight | 70.0 ± 3.8 (12) | 40.2 ± 1.2*** (12) | 41.0 ± 0.2 (11) | 4.1 ± 0.2 (3) |
| | | | | | 4.0 ± 0.1 (6) |
| | Water content | 3.9 ± 0.3 (13) | 4.1 ± 0.2 (11) | 3.0 ± 0.1 (2) | 0.60 ± 0.09 (2) |
| | | | | | 0.60 ± 0.09 (2) |
| | DNA | 0.66 ± 0.19 (12) | 0.62 ± 0.10 (12) | 0.62 ± 0.10 (2) | 0.62 ± 0.09 (2) |
| | | | | | 0.62 ± 0.09 (2) |
| | Protein | 13.9 ± 1.2 (11) | 12.6 ± 1.1 (11) | 10.3 ± 1.8** (3) | 9.7 ± 0.9*** (6) |
| Skin | Wet weight | 2.7 ± 0.13 (12) | 2.1 ± 0.08*** (12) | 2.9 ± 0.19*** (6) | 1.9 ± 0.08*** (5) |
| | | | | | 2.3 ± 0.08*** (6) |

a Weight expressed in grams.

b Water content/unit dry weight.

c Detergent-soluble protein.

Table IV
Crosses Igf2m+/− × K10sIgf2r+/+; parameters of organs not expressing the K10sIgf2r transgene

For values and symbols see legend of Table III.

| A | B | C | D |
|---|---|---|---|
| | Wild type | Igf2m+/− | K10sIgf2r+/+ (Kipps) | K10sIgf2r+/+/*Igf2m+/− (Kipps) |
| | | | | |
| Liver | Wet weight | 1209.9 ± 59.8 (13) | 820.2 ± 20.1*** (13) | 919.6 ± 21.9*** (5) | 716.3 ± 18.1*** (7) |
| | | | | | 843.3 ± 17.3 (6) |
| Kidneys | Wet weight | 322.1 ± 16.8 (14) | 226.6 ± 15.0*** (14) | 359.6 ± 45.6*** (6) | 190.0 ± 12.0*** (7) |
| | | | | | 220.0 ± 10.9** (6) |
| Dry weight | 83.5 ± 6.5 (13) | 50.4 ± 7.9*** (10) | 74.8 ± 6.9*** (7) | 65.5 ± 5.0*** (6) |
| Water content | 3.1 ± 0.4 (11) | 3.2 ± 0.4 (10) | 2.9 ± 0.2 (3) | 2.9 ± 0.3 (6) |
| DNA | 1.72 ± 0.10 (12) | 1.70 ± 0.17 (12) | 1.70 ± 0.20 (2) | 1.80 ± 0.22 (6) |
| Protein | 22.8 ± 3.0 (11) | 18.0 ± 3.0 (11) | 22.6 ± 2.9 (3) | 22.0 ± 2.7 (6) |
| Heart | Wet weight | 121.9 ± 5.1 (14) | 84.5 ± 3.2*** (14) | 128.57 ± 11.8* (6) | 83.4 ± 4.0*** (5) |
| | | | | | 86.5 ± 8.1*** (6) |

a Water content/unit dry weight.

c Detergent soluble protein.

organs examined, whereas no change was observed in water content relative to dry weight, DNA or detergent-soluble protein content (columns B versus column A, Tables III and IV).

K10sIgf2r+/− mice showed a reduction of the wet weight of the stomach, cecum, and colon ranging from 14 to 30% compared with wild type. The skin was not affected, and the liver was the only organ not expressing the transgene that showed a reduction in size (column C in Tables III and IV). The comparison between Igf2m+/− and K10sIgf2r+/− transgenics (Kipps line) with the genetically manipulated element in different mice revealed that the wet weights of organs in the alimentary canal and liver did not differ significantly in the two groups (column B versus C, Tables III and IV). Body weight and wet weights of the skin, kidneys, and heart were significantly higher in Kipps than Igf2m+/− (column B versus column C, Tables III and IV).

Igf2m+/− mice expressing the K10sIgf2r transgene (Igf2m+/−/Kipps and Igf2m+/−/Krishna) showed a body weight similar to Igf2m+/− mice and significantly lower than that of wild type or Kipps (Table III). Among the organs expressing the K10sIgf2r transgene, the wet and dry weights of cecum and colon did not significantly differ in any of the comparisons involving the following four groups: Igf2m+/− mice, Kipps single transgenics, and the double transgenics Igf2m+/−/Kipps or Igf2m+/−/Krishna (columns B–D, Table III). By contrast, the wet weights of the stomach and skin were further decreased in Igf2m+/−/Kipps mice compared with Igf2m+/− and so was the dry weight of the stomach (column D versus column B, Table III). Consistent with the relative levels of K10sIgf2r transgene expression, the decrease in the stomach and skin sizes were more pronounced in Igf2m+/−/Kipps than in Igf2m+/−/Krishna double transgenics (column D in Table III). With the exception of the detergent-soluble protein content in both Igf2m+/−/Kipps and Igf2m+/−/Krishna, none of the other organ parameters was changed compared with Igf2m+/− (column D versus column B, Table III). No major change was observed among organs that did not express the K10sIgf2r transgene (column D versus column B, Table IV). Igf2m+/−/K10sIgf2r+/− mice were born at the frequency expected from the Mendelian law (35/152 or 23%) but showed high mortality during the first 4 weeks of postnatal life (12/19 or 63% dead among Igf2m+/−/Kipps and 3/16 or 19% among Igf2m+/−/Krishna mice). Only 1 Igf2m+/− mouse of 24 was
Fig. 1. Reduction of organomegaly in Blast mice expressing a soluble IGF2R. Cecum and colon from wild type, Blast/Kipps double transgenic, and Blast heterozygote female littermates. Organs were dissected and immediately photographed.

lost before the age of 90 days and wild type or K10sΔIgf2r+/+ heterozygotes were fully viable.

**DISCUSSION**

The membrane form of IGF2R binds IGF-II, retinoic acid, and molecules containing mannose 6-phosphate residues (9–14). This latter group of ligands includes molecules as different in function and structure as the latent form of TGF-β1 and lysosomal enzymes and possibly other still uncharacterized factors (9). Although IGF-II is the only identified ligand of the soluble IGF2R in vivo, it is likely that the two forms of IGF2R share a similar ligand specificity (19). Because of the potential complexity of its binding activity, the biological functions of sIGF2R are difficult to interpret.

We used a genetic approach to discover whether the soluble IGF2R reduces organ size by interacting exclusively with IGF-II. The combined analysis of mice originated by crossing transgensics expressing a soluble IGF2R by mice overexpressing IGF-II or carrying a disrupted paternal (active) allele of the IGF-II gene resulted in a further decrease in the wet weight of the two organs. Furthermore, the wet weights of cecum and colon are not significantly different in Igf2m+/p– and Kipps heterozygotes (Table II and Fig. 2). We conclude that the soluble IGF2R acts exclusively by reducing the bioavailability of IGF-II in these organs.

The second test of the interaction between soluble IGF2R and IGF-II employed the Blast transgenic line, which expressed extra IGF-II in a range of organs similar to those where sIGF2R is abundant in the transgenes (22). At the time of the analysis, the Blast transgene had been bred for over 10 generations onto the 129J/Sv background, and its phenotype differed from the original description in two respects. First, the wet weight increase in the alimentary canal was only mirrored in an increased DNA content in the colon (column B in Table I compared with Ref. 22). Second, there was no change in the fat content of the fat pad corresponding to the IV mammary gland (column B in Table II compared with Ref. 27). The analysis of transgensics overexpressing IGF-II under the control of the same keratin 10 promoter (K10Igf2 transgene, Blast line) and of Blast/Kipps or Blast/Krishna double transgenics revealed that overexpression of IGF-II in the cecum and the colon leads to organomegaly and that the additional expression of sIGF2R in these organs partially reverses the overgrowth phenotype of Blast (Ref. 22, Table I, and Figs. 1 and 2). These data are consistent with the notion that sIGF2R can lower the levels of bioactive IGF-II. Overall, the contribution of the Kipps or Krishna line to the degree of phenotypical changes observed in the crosses discussed in this study correlated with the relative levels of expression of the K10sΔIgf2r transgene in the two lines (21).

**Determination of the Size of the Stomach**—The wet weight of the stomach was decreased in Igf2m+/p– mice by 16% compared with the 30% decrease observed for other organs and the whole body, suggesting that fetal IGF-II has a relatively minor role in the determination of the size of the stomach in the adult (Table III). This conclusion is consistent with the observation that the stomach is relatively unresponsive to the overexpression of IGF-II, despite the high levels of transgenic IGF-II mRNA detected in this organ (Ref. 22, Table I, and Fig. 2).

The expression of the K10sΔIgf2r transgene in a Igf2m+/p– background resulted in a further decrease in the wet weight of the stomach compared with Igf2m+/p– (Table III and Fig. 2). The implication of this result is that sIGF2R controls the size of the stomach by modulating additional factor(s) that are IGF-II-independent. The identity of these factors is uncertain. The only ligands of the membrane form of IGF2R with an established growth regulation activity besides IGF-II are the latent form of TGF-β1 and retinoic acid (1, 13). It is not known whether sIGF2R binds any of the two ligands and whether it is involved in the activation of the latent TGF-β1. The high serum levels of latent TGF-β1 in K10sΔIgf2r transgenics suggest that the soluble IGF2R may act as a reservoir of the growth factor that would then be available for local activation and subsequent growth inhibition (21). The membrane form of IGF2R binds and mediates the angiogenic activity of proliferin (28). sIGF2R may compete for proliferin binding to the cellular form of IGF2R. The soluble receptor may also affect organ size by modulating the activity of mannose 6-phosphate containing enzymes that are involved in the turnover of the extracellular matrix (10, 11).

Surprisingly, the wet weight of the stomach in Igf2m+/p–/Kipps mice is significantly lower than in Igf2m+/p– or Kipps

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3 C. F. Graham, unpublished observations.
heterozygotes, whereas the two latter groups are indistinguishable (Table III). This observation suggests that receptor molecules that are void of IGF-II can interact with other factors more efficiently. *In vitro* experiments indicate that the level of occupancy of the binding site for IGF-II can modulate the binding activity of the mannose 6-phosphate binding site in the membrane form of IGF2R (29, 30). A similar mechanism may function in the sIGF2R molecule.

*IGF2R* mice suffered high mortality (63%) during the first 4 weeks after birth. A possible explanation is that the greatly reduced size of the stomach (57% the size of wild type) resulted in competitive disadvantage in the litter, leading to malnutrition and death. Postnatal mortality was lower (19%) in *Igf2m*/*Krishna* mice, consistent with the less dramatic decrease in stomach size (65% of wild type).

Determination of the Size of the Skin and Uterus—The wet weight of the skin was slightly disproportionate to the rest of the body in *Igf2r*/*−/−* mice compared with wild type (22% wet weight decrease versus 31% body weight decrease, Table III). Skin weight was increased by 60% following IGF-II overexpression (Table I). Surprisingly, the wet weight of the skin was reduced by the expression of sIGF2R in both *Blast* and *Igf2m*/*−/−* backgrounds, although it was unaffected in *K10sΔIgf2r/* transgenics (Tables I and III). During the analysis of the phenotype of *K10sΔIgf2r/* transgenics, we speculated that the lack of responsiveness of the skin to sIGF2R was due to a complex compensatory mechanism preserving the size of the organ (21). Indeed, the data shown in the present study suggest that the size of the skin is controlled by IGF-II (wet weight decrease in *Igf2m*/*−/−*; megalaly in *Blast* and attenuation in *Blast*/Kipps) acting in cooperation with a factor modulated by sIGF2R (reduction in *Igf2m*/*−/−*/Kipps* compared with *Igf2m*/*−/−*). The inability of sIGF2R to attenuate the overgrowth of the uterus in *Blast* is consistent with the low level of expression of the *K10sΔIgf2r* transgene in this organ (21).

**Organ Parameters Affected by IGF-II and sIGF2R**—The total dry weight and water content changed proportionally to the wet weight and to each other in *Igf2m*/*−/−* mice and in organs overexpressing IGF-II or a soluble IGF2R (21). Our result also indicate that in most organs a significant change in dry weight and therefore total water content is accompanied by a significant change in total DNA or detergent-soluble protein contents. Taken together, our data suggest that sIGF2R may act on a set of targets with similar biological activity, one of which is IGF-II.

Another implication is that a major consequence of the alteration of IGF-II levels is a change in fluid and insoluble protein contents. Our data do not unequivocally support the idea that IGF-II causes edema (6–8, 22). The change in fluid content may be secondary to the changes in dry matter and may not involve intercellular fluids. An accumulation of fluid following an increase in the levels of IGF-II may reflect increased vascular permeability and/or change in the vascularization rate with subsequent increased delivery of nutrients from the circulation. The elements that determine what is the “right” organ size are not well characterized. The observation that the growth of the prostate is tightly regulated by the “right” IGF-II concentration suggests that a factor modulated by sIGF2R is needed in this organ to maintain the size of the prostate (31, 32). The membrane and soluble forms of IGF2R, IGF-II, and angiogenic factors such as proliferin may be elements of a system controlling organ size by dictating the rate of angiogenesis. Also, a mechanism must exist by which fetal IGF-II sets the rate at which fluid and insoluble matter are accumulated by tissues and by which this rate is maintained after the levels of circulating IGF-II decline in the adult.

The general conclusions of this work are: 1) the local expres-
sion of a soluble IGF2R reduces the organomegaly induced by excess IGF-II. 2) This reduction involves wet and dry weight in the alimentary canal and skin, but the DNA content is rarely lowered. 3) The soluble IGF2R does not alter the size of the cecum and colon when mice lack IGF-II. This suggests that the soluble IGF2R can reduce organ size through interactions with IGF-II in mice with normal IGF-II levels. 4) The soluble IGF2R reduces the size of the stomach when mice lack IGF-II. This suggests that the soluble IGF2R can also act through an IGF-II-independent pathway (Fig. 2). Further work will be necessary to determine the molecular bases of IGF-II-mediated and -independent activities of sIGF2R and to identify the primary response to IGF-II in the determination of organ size.

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