eliac disease. Out of 12 antibody positive patient 5 gave informed consent for small-bowel biopsy. Of these subjects four had the typical histological features of coeliac disease (Type 3 lesion: flat mucosa and crypt hyperplasia) and one subject had increased intraepithelial lymphocytes (type 1 lesion) suggesting an early stage of the disease [6]. Tissue transglutaminase antibodies were detected in none of 100 healthy controls or 100 patients with newly diagnosed autoimmune thyroid disease (Hashimoto's thyroiditis $n = 23$; Graves' disease $n = 77$).

In conclusion, this study shows that the appearance of autoantibodies to tissue transglutaminase represents a specific marker to identify subjects with silent coeliac disease. These findings indicate that combined detection of IgA-tissue transglutaminase and IgG-tissue transglutaminase antibodies has the potential to overcome the limitations of the conventional endomysium antibody test including the laborious procedure of the assay, problems with assay standardisation and the restriction to the measurement of IgA isotype antibodies. The availability of this novel, non-invasive screening procedure could improve current strategies to identify undiagnosed cases of coeliac disease which is essential to prevent complications such as nutritional deficiencies, small-bowel lymphoma and other severe complications associated with this disease.

Yours sincerely,

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Expression of kinase-inactive mutant insulin receptors does not rescue insulin receptor-deficient mice from perinatal death

Dear Sir,

The insulin receptor mediates insulin action. Mutations of the insulin receptor give rise to insulin resistance. Lack of insulin receptors is lethal in both humans and mice [1], although the phenotypes are different, probably as a result of developmental differences between the two species [2]. Upon insulin binding to the extracellular domain, the insulin receptor undergoes a conformational change that enables the β-subunit to bind ATP and become phosphorylated on several tyrosine residues. This event activates the receptor's kinase toward additional protein substrates and provides the underpinning for the multi-faceted actions of insulin. The pivotal role of the receptor kinase is supported by a host of studies, including naturally occurring mutations of the tyrosine kinase domain in patients with insulin resistance, site-directed mutagenesis of the cloned receptor cDNA, and the determination of the x-ray structure of the receptor's kinase domain. Nevertheless, there exist some lingering reservations as to whether the tyrosine kinase activity is a prerequisite for all insulin actions. These reservations stem from a host of studies, mostly using anti-receptor antibodies that mimic insulin action by binding to the receptor's extracellular domain, but do not activate the receptor tyrosine kinase [3–7].

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To address this issue, we developed transgenic knockout mice that lack endogenous insulin receptors by virtue of being homozygous for a null allele of the receptor gene, but do express a human transgene encoding an ATP binding site mutant insulin receptor (K1030M) [8]. The mutant receptor cannot undergo autophosphorylation. We thought that, if the receptor's kinase activity is not an absolute requirement for insulin action, expression of this transgene should rescue, at least in part, the lethal phenotype due to homozygosity for the insulin receptor mutation.

Mice bearing a null *IR* allele ($IR^{+/-}$) were crossed with IR^{K1030M} transgenic mice to obtain transgenic mice doubly homozygous at the targeted insulin receptor locus and at the transgenic locus ($IR^{-/-,\ K1030M}$). Expression of the human transgene was confirmed by immunoprecipitation of organ extracts and immunoblotting with antibodies against the insulin receptor (Fig. 1). Transgenic knockout mice were born with the expected Mendelian distribution. Their general appearance and body weight were not different from $IR^{-/-}$ mice. Within 48 h of birth, $IR^{-/-,\ K1030M}$ and $IR^{-/-}$ mice developed severe diabetic ketoacidosis, Death ensued within 3–5 days of birth. The slightly longer survival observed in this cross, compared with our previous observations, is probably due to the effect of strain–specific modifier genes and not to the transgene, since it was also observed in non–transgenic knockout mice.

In summary, expression of kinase-defective insulin receptors in mice lacking endogenous receptors does not affect the phenotype we previously reported, with lethal diabetic ketoacidosis. Thus, it is unlikely that insulin action is affected by kinase-defective receptors. There are two potential caveats in the interpretation of these results: firstly, the mutant receptor is expressed at relatively low levels in liver. Since the liver plays a crucial part in energy balance in the perinatal period,

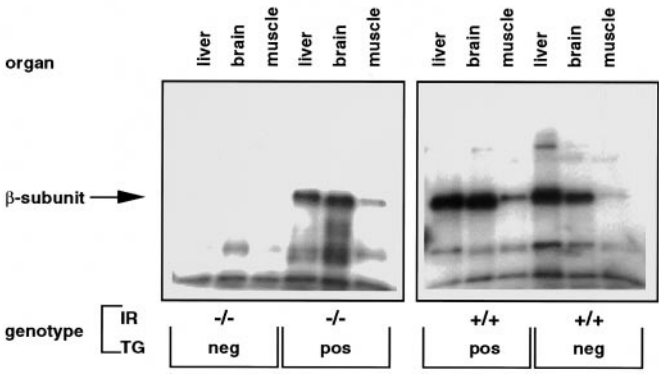

Fig. 1. Immunoblot analysis of insulin receptor expression in newborn mice. Insulin receptors were immunoprecipitated from pooled organ extracts of at least three newborn mice for each genotype using an insulin receptor antibody. The amount of liver and brain extract used was the same in all samples (0.2 mg). The amount of muscle extract varied in the three samples, due to poor recovery from $IR^{-/-}$ and $IR^{-/-,K1030M}$ mice, and is as follows: $IR^{-/-}$: 0.02 mg; $IR^{-/-,K1030M}$: 0.02 mg; WT: 0.15 mg

it is possible that the expression levels of the mutant receptor are not sufficient to rescue the mutant phenotype. Nevertheless, we have observed that mice can survive with insulin receptor levels as low as 10% of normal (Y. Kido and D. Accili unpublished data); so it is unlikely that the failure to rescue the insulin receptor null mice is due to low levels of transgene expression. A more remote possibility, that cannot be addressed in our experimental system, is that the mutant receptor mediates some of the insulin actions in adult life, possibly in tissues that are not generally thought of as insulin targets. Nevertheless, these data provide conclusive evidence that receptor autophosphorylation is required to mediate insulin action.

D. Lauro, Y. Kido, H. Hayashi, Y. Ebina, D. Accili

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Is islet autoimmunity really detectable at birth?

Dear Sir,
It has been shown that Type I (insulin-dependent) diabetes mellitus may start very early in life [1]. In a recent article Lindberg and colleagues reported that islet autoantibodies are not infrequently detected already at birth in children who later develop Type I diabetes, suggesting that beta-cell damage and islet autoimmunity can start in utero [2]. We have measured islet autoantibodies in a similar cohort of children who developed Type I diabetes. From the Type I diabetes Registry of the Lombardy Region 17 patients born between 1991 and 1995 and who presented with diabetes between 1992 and 1996 at less than 5 years of age were identified. All had at least one islet autoantibody at diabetes onset and 14 presented with ketoacidosis. We obtained the blood spot collected during the first week of life for metabolic testing from these 17. Blood spots were also obtained from 37 control neonates born in the same hospital on the same day and with no family history Type I diabetes in the mother and from 20 newborns of mothers with Type I diabetes. Blood spots were extracted and eluate measured for antibodies to glutamic acid decarboxylase (GADA), IA–2 (IA–2A), and insulin (IAA) as described previously [3, 4]. None of the birth blood spots from children who developed diabetes or control children had detectable islet autoantibodies, whereas at least one autoantibody was detected in 7 of 20 children from mothers with Type I diabetes. One of these children, who had IAA and GAD at birth, presumably from the mother's circulation developed Type I diabetes at age 4 years. These data further suggest that islet autoantibodies found at birth are likely to be acquired through transplacental exchange of the mother's immunoglobulin and support other reports [4, 5] which indicate that in utero production of islet autoantibodies is likely to be a relatively rare occurrence.

Yours sincerely,
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