agnosis before the first insulin injection because of the insulinopaenia, high blood glucose load and deranged lipid metabolism, and then increases at later follow up with more normal blood glucose and metabolic control.

In conclusion, childhood Type 1 diabetes seems to be associated with abnormalities in ghrelin secretion. The circulating concentrations are low prior to insulin treatment and responses to meal tests are absent. As ghrelin is involved in the regulation of feeding behaviour and energy homeostasis, abnormalities in ghrelin secretion may play a role for the metabolic balance in diabetic children.

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## References

1. Kojima M, Hosoda H, Matsuo H, Kangawa K (2001) Ghrelin: discovery of the natural endogenous ligand for the growth hormone secretagogue receptor. Trends Endocrinol Metab 12:118–122
2. Casanueva FF, Dieguez C (2002) Ghrelin: the link connecting growth with metabolism and energy homeostasis. Rev Endocr Metab Disord 3:325–338
3. Tschop M, Weyer C, Tataranni PA, Devanarayan V, Ravussin E, Heiman ML (2001) Circulating ghrelin levels are decreased in human obesity. Diabetes 50:707–709
4. Shiiya T, Nakazato M, Mizuta M et al. (2002) Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. J Clin Endocrinol Metab 87:240–244
5. Otto B, Cuntz U, Fruehauf E et al. (2001) Weight gain decreases elevated plasma ghrelin concentrations of patients with anorexia nervosa. Eur J Endocrinol 145:679–683
6. Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS (2001) A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. Diabetes 50:1714–1719
7. Dornonville de la Cour C, Bjorkqvist M et al. (2001) A-like cells in the rat stomach contain ghrelin and do not operate under gastrin control. Regul Pept 99:141–150
8. Caixas A, Bashore C, Nash W, Pi-Sunyer F, Laferrere B (2002) Insulin, unlike food intake, does not suppress ghrelin in human subjects. J Clin Endocrinol Metab 87:1902–1906

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*Observation*

# CD14 triggers autoimmune Type 1 diabetes in the NOD mouse

*To the Editor:* Type 1 diabetes is an autoimmune disease caused by selective destruction of the insulin-producing beta cells. Both, genetic predisposition and non-genetic, environmental factors, are believed to be involved in inducing Type 1 diabetes [1]. Several mechanisms by which environmental factors, such as pathogenic agents, might be involved in the development of autoimmune diseases have been proposed. One mechanism, "molecular mimicry"—has become popular in explaining the loss of tolerance in autoimmune diseases. In this hypothesis an immune response mounted against a foreign determinant cross-reacts with a host determinant, leading to inflammation and autoimmune destruction of the host tissue or organ. Similarities in sequence, structure or epitope-sharing between pathogens and host antigens have all been put forward as mechanisms which might drive this phenomenon [2, 3]. An association of infection with a particular pathogen and the induction of autoimmunity have been shown for reactive arthritis (RA), Guillain-Barre syndrome (GBS) and multiple sclerosis (MS) [4, 5, 6, 7]. Bacteria as potent immunogens express many factors that can act as immune stimulants. A multifunctional receptor CD14, interacts with several cell-wall components of gram-positive and gram-negative bacteria including lipopolysaccharide (LPS) and peptidoglycan. Recent studies suggest that CD14 is not only able to act as an immune receptor for "non-self" components such as LPS but it is also able to interact with "self" components (apoptotic cells) [8]. Since CD14 plays a key role in the regulation of the inflammatory cascade and since it also interacts with "self" components, perhaps there is an involvement of CD14 in the development of Type 1 diabetes in the non-obese diabetic (NOD) mouse model. To this end we have used classic genetic procedures to generate a NOD congenic line carrying a targeted deletion of the *CD14* gene.

Our experiments where carried out in accordance with the rules for animal care of the Ministry of Nutrition, Agriculture and Forestry of the German government and were approved by the Institution's animal care and use committee. *CD14*-deficient male mice (backcrossed into the C57BL/6J, a gift from Dr. D. Golenbock (Boston Medical and Boston University School of Medicine, Boston, Mass., USA) were crossed with NOD female mice (M&B A/S, Denmark) to produce F1 hybrids which were backcrossed onto NOD background. In each backcross generation mice which were heterozygous for *CD14* deficiency (*CD14*+/–) were selected using primers for *CD14* (F: 5′-CCAAGTTTTAGC GCTGCGTAAC-3′; R: 5′-GCCA-GCCAAGGATACATAGCC-3′) and for the *neo*-gene of *CD14*–/– (F: 5′-GTCAAGACCGACCTGTCCGG-3′;R: 5′-TC-

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**Table 1.** Frequency and age at onset of diabetes in NOD vs NOD.CD14–/– mice

| Traits | NOD | | NOD.CD14–/– | | NOD vs. NOD.CD14–/– *p* value | |
|---|---|---|---|---|---|---|
| Mice | Males | Females | Males | Females | Males | Females |
| Diabetes Frequency | 19%[a] (12/65) | 67% (51/76) | 3%[a] (1/35) | 24% (8/33) | 0.022 | <0.0001 |
| Age at onset (days) | 153±40[a] | 128±33 | 146 | 204±55 | | <0.0001 |
| (Min-max) | 99–216 | 87–210 | 146 | 138–270 | | |

[a] Significant sex differences at 1%

GCCGCCAAGCTCTTCAGC-3′). These heterozygous animals were repeatedly backcrossed onto NOD. After seven backcross generations heterozygous animals were intercrossed and *CD14–/–* were selected to found the congenic NOD.CD14–/– strain. The NOD background identity was checked and confirmed by 171 microsatellite markers on 19 autosomes (kindly carried out by G. Brockmann, Research Institute for the Biology of Farm Animals, Dummerstorf, Germany) and by skin grafting. We observed 68 NOD.CD14$^{-/-}$ (N8F1, 35 males/33 females) and 141 NOD mice (65 male:76 female) for diabetes occurrence up to the age of 270 days. All animals were screened for glucosuria twice a week using test tapes (Diabur-Test 5000, Boehringer, Mannheim, Germany) starting at the age of 60 days. Diabetes was diagnosed on the basis of glucosuria followed by measuring blood glucose concentrations above 15 mmol/l on two consecutive days. Both strains were kept in the same animal room under conventional holding conditions in Macrolon cages (Size 2, Ehret, Emmendingen, Germany) and were free of major mouse pathogens (MHV, Reo3, TMEV, PVM, Sendai, MVM, Mycoplasma pulmonis). They had food (Ssniff M, Soest, Germany) and water ad libidum and were maintained on a 12-h light and dark cycle (5 a.m./5 p.m.). Data were evaluated using the statistical analysis system SPSS (SPSS, Chicago, Ill., USA). The age at disease onset is given as means ± SD. Differences between NOD and NOD.CD14-/- in diabetes frequency were analysed by two-tailed Chi square test and mean age at onset was compared by one-way ANOVA analysis.

We found that the frequency of diabetes is significantly decreased in *CD14*-deficient female and male NOD mice compared with their parental strain, NOD (Table 1). In addition, *CD14*-deficient diabetic mice developed symptoms considerably later than the NOD animals. The presence or absence of CD14 did not affect the diabetes preponderance in NOD female mice.

Whether the involvement of CD14 requires interaction with LPS or with some host component remains to be elucidated. In the absence of CD14 the signalling pathway through nuclear factor kappa beta could be limited and thus lead to a reduced expression of immune response genes like *cyclooxygenase-2*. This notion is supported by the fact that blocking of cyclooxygenase activity with indomethacin reduces diabetes incidence in female NOD-mice [1]. However, these results indicate that there is an association between a central component of the innate immune system and diabetes development in the NOD mouse.

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## References

1. Beyan H, Buckley LR, Yousaf N, Londei M, Leslie RDG (2003) A role for innate immunity in type 1 diabetes? Diabetes Metab Res Rev 19:89–100
2. Tsuchiya N, Williams RC Jr (1992) Molecular mimicry or reality? West J Med 157:133–138
3. Robles DT, Eisenbarth GS (2001) Type 1A Diabetes induced by infection and immunization. J Autoimmunity 16:355–362
4. Herrath MG von, Holz A, Homan D, Oldstone MB (1998) Role of viruses in type 1 diabetes. Semin Immunol 10:87–100
5. Granfors K, Toivanen A (1986) IgA-anti-yersinia antibodies in yersinia triggered reactive arthritis. Ann Rheum Dis 45:561–565
6. Bohemen CG van, Grumet FC, Zanen HC (1984) Identification of HLA-B27M1 and -M2 cross-reactive antigens in Klebsiella, Shigella and Yersinia. Immunology 52:607–610
7. Moran AP, Prendergast MM (1998) Molecular mimicry in *Campylobacter jejuni* lipopolysaccharides and development of Guillain-Barre syndrome. J Infect Dis 178:1549–1551
8. Devitt A, Moffatt OD, Raykundalia C, Capra JD, Simmons DL, Gregory CD (1998) Human CD14 mediates recognition and phagocytosis of apoptotic cells. Nature 392:505–550

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