brane growth and ultrafiltration is unknown (4). Since VEGF, also known as vascular permeability factor (VPF), is mainly upregulated by hypoxia (1), we speculated that it is closely related to the pathophysiology of rubeotic glaucoma, as well as that of PDR.

We determined VEGF levels in aqueous humor, obtained with informed consent from patients with diabetic retinopathy complicated by rubeotic glaucoma, with a sensitive enzyme-linked immunosorbent assay (ELISA) method as we previously described, with slight modifications (5). Extremely elevated levels of VEGF (1,404.1 ± 172.7 pg/ml, mean ± SD, $n = 9$, $P < 0.001$) were observed in the aqueous humor of diabetic patients with rubeotic glaucoma compared with that of patients with PDR (95.9 ± 29.1 pg/ml, $n = 12$) (background/preproliferative diabetic retinopathy patients, 41.8 ± 25.4 pg/ml, $n = 9$; diabetic patients without retinopathy, 16.1 ± 14.5 pg/ml, $n = 8$). Our data suggest that VEGF may play a central role in the pathophysiology of rubeotic glaucoma. Inhibition of VEGF may be a new approach to the treatment of rubeotic glaucoma.

HIROHITO SONE, MD
YUKICHI OKUDA, MD, PHD
YASUSHI KAWAKAMI, MD, PHD
MITSUYA HANATANI
HIDEO SUZUKI, PHD
TADAHIKO KOZAWA, MD, PHD
SACHIKO HONMURA, MD, PHD
KAMEJIRO YAMASHITA, MD, PHD

From the Department of Endocrinology and Metabolism (H.S., Y.O., Y.K., K.Y.) and the Department of Ophthalmology (S.H.), University of Tsukuba; the Kozawa Hospital (T.K.); and the Bioscience Research Department (M.H., H.Su.), Tsukuba Research Laboratory, Toagosei Co. Ltd., Tsukuba, Japan.

Address correspondence to Yukichi Okuda, MD, PhD, Department of Endocrinology and Metabolism, University of Tsukuba, Tsukuba, 305 Japan.

References

1. Ferrara N, Houck K, Jakeman L, Leung DW: Molecular and biological properties of the vascular endothelial growth factor family of proteins. *Endocr Rev* 13:18–32, 1992
2. Adamis AP, Miller JW, Bernal MT, D'Amico DJ, Folkman J, Yeo TK, Yeo KT: Increased vascular endothelial growth factor levels in the vitreous of eyes with proliferative diabetic retinopathy. *Am J Ophthalmol* 118:445–450, 1994
3. Aiello LP, Avery RL, Arrigg PG, Keyt BA, Jampel HD, Shah ST, Pasquale LR, Thieme H, Iwamoto MA, Park JE, Nguyen HV, Aiello LM, Ferrara N, King GL: Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. *N Engl J Med* 331:1480–1487, 1994
4. Rehak J: Neovascular glaucoma and intraocular pressure. I. Pathogenesis of increased intraocular pressure and therapy: a review of historical and current therapeutic modalities. *Acta Univ Palacki Olomuc Fac Med* 133:71–73, 1992
5. Hanatani M, Tanaka Y, Kondo S, Omori I, Suzuki H: Sensitive chemiluminescence enzyme immunoassay for vascular endothelial growth factor/vascular permeability factor in human serum. *Biosci Biotechnol Biochem* 59:1958–1959, 1995

HbA_{1c} Assay on Capillary Blood Sample Collected at Home: A Reliable Method

We previously reported the stability of capillary blood samples for HbA_{1c} assay for 72 h at room temperature by the immunochemical method using a DCA 2000 analyzer (Bayer Diagnostic, Milan, Italy) (1,2). This result suggested to us to assess the stability and validity of HbA_{1c} assessed on capillary blood samples that were collected at home by diabetic patients and mailed to our clinic. We have designed a study to assess the effects of mailing on capillary blood samples that are used to assess HbA_{1c} by DCA 2000 and the patient's ability to collect the samples at home.

Capillary blood samples were collected from 31 patients (11 males and 20 females) with mean age of 15.8 ± 3.3 years and mean duration of diabetes of 7.3 ± 4.0 years. The mean HbA_{1c} assessed from capillary blood samples taken during clinic visits was 9.0 ± 1.5%, and the mean HbA_{1c} assessed from capillary blood samples that were collected during the same visits and mailed to the hospital was 9.1 ± 1.4 %. Correlation between the groups of samples was $r = 0.905$ and $P < 0.0001$. Mailing time was a median 2.8 (range, 1–7) days. Mailing time was within 3 days for 18 samples, and 13 samples were received after 4.8 ± 1.3 days. However, concerning the change in HbA_{1c} (ΔHbA_{1c}; HbA_{1c} values assessed during the clinic visit minus the HbA_{1c} values assessed on mailed samples), we did not observe any significant difference between samples received before and after 3 days (0.05 ± 0.5 vs. −0.15 ± 0.3%). In our region, the mean temperature during wintertime is 2.8°C, and the mean temperature during summertime is 24.5°C. Twenty-one samples were mailed during winter and ten samples during summer, but we did not observe any difference regarding ΔHbA_{1c} mailed during winter and summer (−0.02 ± 0.6 vs. 0.06 ± 0.3%).

We have assessed the ability to collect capillary blood samples for HbA_{1c} determinations on a subgroup of 18 patients with a mean age of 16.9 ± 2.1 years and a mean diabetes duration of 8.6 ± 3.7 years. We compared HbA_{1c} values assessed from capillary blood samples collected during visits and mailed to hospital with HbA_{1c} values that were assessed from samples collected at home by the patients on the same day of the visits and mailed to hospital. The mean HbA_{1c} assessed from mailed capillary blood samples collected during the clinic visit was 9.7 ± 1.6, and the mean HbA_{1c} assessed from capillary blood samples collected at home by the patients was 9.7 ± 1.5%. Correlation between the groups of samples was $r = 0.942$ and $P < 0.0001$.

In conclusion, the assessment of HbA_{1c} levels from a capillary blood sample collected by patients at home and mailed to hospital within 7 days is a stable, reliable, and easy method. This procedure could be useful in patients with poor metabolic control who require frequent HbA_{1c} assessments but who do not agree with frequent clinic visits, as is often the case during adolescence (3). However, the advantages of frequent HbA_{1c} assessment to improve metabolic control should be tested.

EMILIO R.M. BOGNETTI, MD
ALEXA ZOJA, MD
ANNA COGLIARDI, MD
FRANCO MESCHI, MD
GIUSEPPE CHIUMELLO, MD

From the Department of Pediatrics, Scientific Institute San Raffaele, University of Milan, Italy.

Address correspondence and reprint requests to Emilio Bognetti, MD, Scientific Institute H San Raffaele, Department of Pediatrics, Endocrine Unit, via Olgettina 60, 20132 Milano, Italy.

References

1. Bognetti E, Zoja A, Ciralli F, Chiumello G: HbA_{1c} determination on capillary blood

sample: validity, stability, and potential usefulness. *Diabetes Care* 18:1305–1306, 1995
2. Ng RH, Sparks KM, Hiar CE: Rapid automated immunoassay system for measuring HbA_{1c} by using precalibrated unitized reagent cartridges. *Clin Chem* 38:1647, 1992
3. Amiel SA, Sherwin RS, Simonson DC: Impaired insulin action in puberty: a contributing factor to poor glycemic control in adolescents with diabetes. *N Engl J Med* 315:215–219, 1986

Neonatal Diabetes and DQB1 Typing

HLA haplotypes typical for IDDM may be present in patients with neonatal diabetes, and HLA-DR4 and -DR3 increase the likelihood of the permanent form of diabetes (l). Serological HLA-DR3 and -DR4 typing is thought to support the early differentiation between the permanent and transient types of congenital diabetes. However, HLA-DR alleles lack specificity in the prediction of developing the disease, being present in 40% of the nondiabetic population (2). Currently, new high-risk genetic markers are available. Restriction fragment length polymorphism analysis started to implicate the HLA-DQ molecule more than HLA-DR in IDDM susceptibility. Alleles encoding for an amino acid, different from aspartate at position 57 (non-Asp_{57}) of the β-chain of the DQ molecule (HLA-DQB1), have been shown to be more strongly and positively associated with the disease (3).

To our knowledge, there have been no previous reports of HLA-DQB1 typing in neonatal diabetes.

We retrospectively performed HLA-DQB1 typing on three patients with neonatal diabetes ( ). Two out of three (cases 1 and 3) presented the transient form, and no recurrence occurred within 9 and 2 years, respectively, while case 2 developed the permanent form. All patients persistently lacked autoimmune IDDM-related autoantibodies. In particular, the patient with the permanent form appeared homozygous for highly susceptibility alleles (non-Asp_{57}/non-Asp_{57}), while alleles coding for aspartate residue (Asp_{57}), which are regarded as protective, were detected in the remaining two.

Our data support the hypothesis that DQB1 typing may be useful to predict the permanent form of neonatal diabetes. The wide variety of clinical presentation and outcome suggests that more than one mechanism may be implicated in the etiology of this condition. Although serological evidence of autoimmunity has never been reported, the clinical course of permanent neonatal diabetes is indistinguishable from IDDM, and we should be careful to consider them two distinct entities. Furthermore, neonatal diabetes may be considered a high-risk condition to develop IDDM.

In our opinion, HLA-DQB1 more than DR3/DR4 typing in patients with the permanent form or later recurrence may represent a challenging clue in understanding if and how neonatal diabetes is related to IDDM.

MASSIMO MAZZELLA, MD
MARIO COTELLESSA, MD
WANDA BONACCI, MD
CLAUDIO BRUNO, MD
GIOVANNI SERRA, MD

From the Department of Child Heath and Neonatal Medicine (M.M., W.B., G.S.) and the Diabetology Care Unit (M.C., C.B.), Department of Pediatrics, Gaslini Children's Hospital, Genova, Italy.

Address correspondence and reprint requests to Massimo Mazzella, MD, Department of Child Health and Neonatal Medicine, Gaslini Children's Hospital, Largo G. Gaslini 5, I-16148 Genova, Italy.

Table 1—*Case comparisons*

Patient	Duration of Hyperglycemia	DQB1 typing	Outcome	Duration of follow-up
Case 1	50 days	*0201,02/0301 (non-ASP/ASP)	Normoglycemia	9 years
Case 2	Permanent	*0201,02/0501 (non-ASP/non-ASP)	IDDM	6 years
Case 3	35 days	*0301/0301 (ASP/ASP)	Normoglycemia	2 years

References
1. Von Muhlendal KE, Herkenhoff H: Long-term course of neonatal diabetes. *N Engl J Med* 333:704–708, 1995
2. Cudworth AG, Wolf E: The genetic susceptibility to type I (insulin- dependent) diabetes mellitus. *J Clin Endocrinol Metab* 11:389–408, 1982
3. Todd JA, Bell JL, McDevitt HO: HLA-DQ-beta gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. *Nature* 329:559–563, 1987

The Prevalence of Anti-Bovine Serum Albumin Antibodies in Japanese Children With IDDM

Bovine serum albumin (BSA) in cow's milk proteins has been suggested as a possible trigger molecule in the development of IDDM in Caucasians, especially Finns (1–3). However, the implications of BSA in the pathogenesis of IDDM are controversial even in Caucasians (4,5) and have been addressed in limited studies in other ethnic groups, including Japanese (6). The remarkably low incidence of IDDM in Japanese children (1.5–2.0 per 100,000 individuals) may be attributed to differences in environmental and/or genetic factors. Cow's milk formula has been becoming increasingly popular for infant feeding in Japan. Therefore, it is of major interest whether BSA may be involved in the pathogenesis of IDDM in Japanese children, whose genetic background is quite different from that of Caucasians (7). On the other hand, a high incidence of antibodies to GAD (GADAb) has been demonstrated in Japanese IDDM patients as well as in Caucasians (8,9).

We examined the prevalence of antibodies to BSA (BSAAb), ABBOS (a 17–amino acid peptide, position 152 to 168 of BSA), and GAD in sera from Japanese children with IDDM and other diseases, as well as the prevalence in normal children (Table 1). The specific elevation of BSAAb in sera from IDDM patients was demonstrated first by Karjalainen and colleagues (1,2) using a newly developed particle-concentration fluoroimmunoassay (PCFIA). They reported that the sensitivity and specificity of PCFIA in detecting disease-associated BSAAb were much higher