Differential β-Cell Response to Glucose, Glucagon, and Arginine During Progression to Type I (insulin-dependent) Diabetes Mellitus

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Acute insulin responses to glucose (AIRG), glucagon (AIRGln), and arginine (AIRArg) were evaluated prospectively in nine subjects positive for islet-cell antibodies (ICAs) who later progressed to type I diabetes or impaired glucose tolerance (IGT) (progressors), 64 ICA-positive subjects at risk who did not develop type I diabetes, 365 ICA-negative relatives of diabetic patients who also remained free of the disease, and 89 control subjects. Seven progressors already had a low AIRG at entry into the study, and the other two became low responders 3 to 9 months before diabetes or IGT, with a progressive decline of AIRG over serial intravenous (IV) glucose tolerance tests. At entry into the study, the group of progressors displayed lower AIRG, AIRGln, and AIRArg than the other three groups ($P < .001$). However, AIRArg was less altered than AIRG. During the course of the prediabetic phase, there was a progressive decline in AIRG and AIRGln analyzed as a function either of time ($P < .006$) or of basal glycemia ($P < .05$), ie, two different ways of estimating worsening of the disease process. Conversely, there was no significant decrease in AIRArg with time or with increasing basal glycemia, so that AIRArg was not totally blunted in these prediabetic subjects even a few months before the onset of diabetes. The persistence of a substantial response to arginine, ie, higher than the fifth control percentile, even at a late stage, was confirmed in five of nine diabetic patients tested either at onset of the disease or during non–insulin-receiving remission. Whereas AIRG deteriorates during prediabetes, AIRArg appears to be less altered with time and increased basal glycemia, remaining substantial even at the onset of the disease. This reinforces the supposition that the prediabetic state may be associated with a glucose-specific β-cell functional abnormality in addition to a decreasing β-cell mass.

TYPE I (insulin-dependent) diabetes mellitus is a genetically programmed autoimmune disease resulting from the selective destruction of pancreatic β cells.[1] Clinical onset of the disease is preceded by a latency period detectable by immune markers. Cytoplasmic islet-cell autoantibodies (ICAs) are the best operational predictive markers in subjects at risk.[2-4] Their predictive value is enhanced by the detection of insulin autoantibodies (IAA),[5] antibodies to the 64K islet antigen,[6-9] or glutamic acid decarboxylase (GAD) antibodies,[10-12] or by the staining pattern of ICAs.[13-18] Metabolic abnormalities can also be detected during the prediabetic period. Slightly elevated basal blood glucose values may indicate imminent onset of the disease.[19] Impaired oral glucose tolerance may precede onset of the disease in identical twins of type I diabetic patients.[20] Progressive loss of the acute insulin response (AIR) to intravenous (IV) glucose (AIRG) may be an early event,[21-25] as well as a glucose failure to potentiate arginine-induced insulin release[26] or a loss of regular oscillatory insulin secretion.[27] Studies on AIR to nonglucose stimuli in pre–type I diabetes are still incomplete. Decreased AIRG may coexist with subnormal AIRs to other stimuli in prediabetic humans,[28] in islets of diabetes-prone non-obese diabetic (NOD) mice,[29] and in BioBreeding Worcester (BB/W) rats.[30] Alterations of glucose and glucagon-stimulated insulin release have been described in ICA-positive relatives of type I diabetic patients.[31,32] We previously suggested that AIR to arginine (AIRArg) was less impaired than AIRG and AIR to glucagon (AIRGln) in prediabetic subjects.[33] The conclusions of these few previous studies have hazards in extrapolation because sample sizes were small and evaluation was cross-sectional. Further studies on responsiveness to different secretagogues during pre–type I diabetes are thus needed because they may help to clarify β-cell functional state. Do responses to different stimuli decrease simultaneously during prediabetes? The answer to this question will require long-term follow-up studies with serial metabolic tests. In the present study, we performed a prospective evaluation of serial IV AIRG, AIRGln, and AIRArg in subjects at risk for type I diabetes. The results were analyzed as a function of time and basal glycemia considered as indexes of worsening of the disease process. Moreover, in view of the few subjects who develop diabetes in such studies and the need to substantiate the description of metabolic status at the end point of the prediabetic phase, we evaluated the same metabolic parameters in recent-onset type I diabetic patients.

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Submitted March 6, 1995; accepted July 2, 1995.

Supported by grants from INSERM, Caisse Régionale d'Assurance Maladie de Loire-Atlantique et de Bretagne, Direction Régionale de l'Action Sanitaire et Sociale des Pays de Loire, and Délégation à la Recherche Clinique.

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0026-0495/96/4503-0005$03.00/0

SUBJECTS AND METHODS

Subjects

Nine ICA-positive subjects (five females and four males aged 11 to 32 years) who later progressed to type I diabetes (n = 8) or impaired glucose tolerance ([IGT] n = 1) were studied. Five (cases no. 1 to 5) were ICA-positive relatives recruited from our prospective evaluation of 708 first-degree relatives of type I diabetic patients.[18,25] Four other ICA-positive individuals (cases no. 6 to 9)

without a family history of diabetes were included because transient basal hyperglycemia (> 6.1 mmol/L) and had been detected during medical examination or presurgical screening. In these latter subjects, fasting glycemia had returned to normal by the time of the tests. None of these subjects at risk displayed diabetes or IGT when the initial serum sample was drawn.

They were compared with (1) 64 subjects classified as ICA-positive on human pancreas, 41 of whom (19 females and 22 males; mean age, 17 ± 1 years; range, 3 to 45) were recruited in our family study (prevalence rate of ICA, 6.5%).[16,18] Twenty-three ICA-positive healthy schoolchildren from the French background population (seven boys and 16 girls; mean age, 13 ± 0.5 years; range, 8 to 18) without a family history of diabetes were obtained from a cohort of 1,278 children (ICA prevalence rate, 1.8%) initially screened in 1989 at the Institut Régional de Santé (Tours, France). The ICA status of this cohort was previously published[18]; (2) 365 ICA- and IAA-negative first-degree relatives (176 females and 189 males; mean age, 19 ± 0.5 years) who were recruited in our family study; and (3) 89 healthy adult volunteer controls who had no personal or familial history of diabetes or autoimmune disease.

Nine type I diabetic patients were also tested either at onset (n = 4), before insulin was started, or during non–insulin-receiving remission (n = 5). Patients at onset were ketotic but had no acidosis, with basal glycemia varying between 11.0 and 22.0 mmol/L. Complete remission was defined as good metabolic control (intended to achieve fasting blood glucose < 7.8 mmol/L, postprandial blood glucose < 11.1 mmol/L, and hemoglobin A_{1C} < 7.5%) in the absence of insulin treatment.

Subjects were taking no medication and had a diet of 250 g carbohydrate for 3 days before the tests. Personal or parental informed consent was obtained, and the study was approved by the ethics committee.

Antibody Analysis

ICAs were detected in a blinded manner by indirect immunofluorescence on sections of human frozen pancreas, as previously described.[16-18] Results are expressed in Juvenile Diabetes Foundation (JDF) units. Subjects were classified as ICA-positive if they had at least two positive samples (≥ 5 JDF units). One ICA-positive internal standard and reference sera from international workshops were included. In the Eighth International ICA Workshop, our laboratory had 100% sensitivity and 100% specificity in blinded analysis of test serum samples, with a detection limit of 2.5 JDF units.

ICAs on mouse pancreata were detected by indirect immunofluorescence, as previously described.[16-18] Sections were incubated with serially diluted human sera and developed with fluoresceinated rabbit $F(ab')_2$ fragments of antihuman IgG (H + L; Bioatlantic, Nantes, France). Results are expressed as end-point titers. In each assay, one positive internal standard (80 JDF units on human pancreas) and one negative sample were included as quality controls. The negative control was read as negative on 50 occasions. The positive control was always read as positive, with a mean end-point titer of one in 64 and an interassay variation of 0.5 titration steps.

Antibodies to the 64K islet antigen were determined as previously described[17,18] using a modification of the immunoprecipitation method of Christie et al.[9] Islets were isolated from rat pancreata. Extracts labeled with ^{35}S-methionine (Amersham, UK) were precipitated with the test serum, and the immune complexes were bound to protein A–Sepharose (Pharmacia, Uppsala, Sweden). After separation by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, all fluorographs were analyzed independently by two observers unaware of sample identity. Negative and positive control sera were included in each experiment.

Antibodies to GAD were assayed in a blinded manner as previously described[17,18] by determining enzyme activity immunotrapped by sera from brain extracts.[10-12] Rat brain extracts from adult Wistar rats were incubated with test serum. Immune complexes isolated on protein A–Sepharose were incubated with [1-^{14}C]glutamic acid, and $^{14}CO_2$ release was then measured by scintillation counting. GAD activity was calculated as the percentage of activity trapped by the same standard antibody-positive control serum used in analyses of 64K antibodies. Sera were regarded as positive if activity was greater than 6%, ie, greater than 3 SD of the activity in sera from 82 control subjects (mean ± SD, 3% ± 1%). In the First International GAD Antibody Workshop, our laboratory had 100% sensitivity and 100% specificity in blinded analysis of test serum samples.[34]

Sera were tested for the presence of IAA using the ability to bind monoiodinated ^{125}I-Tyr-A-14 human insulin (specific activity, 250 μCi/μg; Novo, Copenhagen, Denmark). Interassay and intraassay coefficients of variation were 15% and 10%, respectively. A positive internal standard sample was included in each assay. A positive result was defined as a value greater than 1.3%, ie, greater than 3 SD above the mean binding of control sera (0.94% ± 0.12%). These control sera displayed normally distributed values. Sensitivity of the assay was 0.15%. In the Fourth International IAA Workshop, our laboratory had 100% sensitivity and 100% specificity in blinded analysis of test serum samples.

Metabolic Studies

As previously described, AIRG, AIRArg, and AIRGln were evaluated in this order (at 2-day intervals) in the morning after a 12-hour overnight fast.[25,33] To evaluate AIRG, catheters were inserted into both antecubital veins, and 30% glucose 0.5 g/kg body weight was injected over 2 minutes ± 5 seconds. To evaluate AIRArg, 5 g arginine (Veyron, Marseille, France) was diluted in 50 mL of a 0.9% NaCl solution and injected over 1 minute ± 5 seconds. To evaluate AIRGln, 2 mg porcine glucagon (Novo) was diluted in 60 mL of a 0.9% NaCl solution and injected over 1 minute ± 5 seconds. For children, the doses were 1 g arginine/6 kg body weight and 0.02 mg glucagon/kg. Doses of glucagon and arginine used here have been reported to induce maximal insulin release.[35,36] Previous studies[35,37] have shown that AIRs to these two secretagogues, injected as a 30-second to 1-minute bolus, peak during the 2- to 4-minute period.

Blood samples were taken 10 minutes before each bolus and 1 and 3 minutes after the end of the injection. Each sample was drawn in less than 15 seconds and kept on ice until centrifugation. Plasma was then frozen at −20°C. Blood glucose level was measured by a glucose oxidase method. Basal and stimulated immunoreactive insulin (IRI) concentrations were measured by radioimmunoassay (SB-INSI-5; International CIS, Saclay, France) using ^{125}I-porcine insulin, human insulin as a standard, and guinea pig antiserum to human insulin. Separation was achieved with polyethylene glycol. Undiluted plasma leading to IRI values greater than 60 μU/mL was further diluted until it was within the linear portion of the standard curve. Assay sensitivity was 2.5 μU/mL, and intraassay and interassay coefficients of variation were less than 10%. In blinded analysis of test serum samples, a strong correlation was found with the laboratory reference elicited by the Islet Cell Antibody Register Users Study ($P < .0001$, $r^2 = .97$, $y = 0.6x - 0.9$).

Previous studies reported interindividual and intraindividual variabilities of AIRG, AIRGln, and AIRArg in adult healthy control subjects.[38-41] As we previously reported,[25] a value less than the first percentile of age-related ICA- and IAA-negative relatives defined a low AIRG (prepubertal children, 20 μU/mL; Tanner's stages 2 to 5, 37; adults, 20). The overall ranking of percentiles in

365 ICA- and IAA-negative subjects led to a first percentile value of 27 μU/mL. "Age-related" changes of AIRG have also been reported by others.[41] First percentiles for AIRGln and AIRArg were, respectively, 15 and 31 μU/mL in the population of ICA-negative control subjects.

Oral glucose tolerance tests (75 g glucose load for adult subjects and 1.75 g/kg for children) were performed. IGT and diabetes mellitus were defined according to the criteria of the World Health Organization.

HLA Typing

Lymphocytes from blood samples were typed for HLA-DR antigens by the two-color immunofluorescence technique.

Statistical Analysis

AIRG, AIRGln, and AIRArg were calculated as the sum of 1- and 3-minute insulin values and log-transformed for purposes of statistical analysis because of abnormal distributions of insulin values. Differences were evaluated by Student's t test or the nonparametric Mann-Whitney test. Significance of correlations between AIRG, AIRGln, AIRArg and delay to diabetes or basal glycemia were tested by linear regression analysis. Differences were considered significant when P was less than .05.

RESULTS

At entry into the study, mean AIRGs were similar in ICA-positive patients, ICA-negative relatives, and control subjects (134 ± 13, 128 ± 4, and 116 ± 9 μU/mL, respectively; Fig 1). No statistical differences were observed between AIRG, AIRGln, and AIRArg within each of these three groups.

Within a follow-up period of 7 years, four ICA-positive relatives progressed to type I diabetes and a fifth one developed IGT (Fig 2). Pertinent characteristics of these subjects (cases no. 1 to 5) are indicated in Table 1. These five patients were HLA-DR 3 and/or 4. All five displayed GAD antibodies, and four had 64K antibodies. All but one had IAA. Three (cases no. 1, 2, and 5) had low AIRG at entry into the study (Fig 2). The fourth relative (case no. 3) displayed normal AIRG at entry but became a low responder 3 months before the onset of diabetes. The fifth relative (case no. 4) initially displayed normal AIRG, which decreased to less than the first age-related percentile 9 months before IGT. A progressive decline of AIRG over serial IV glucose tolerance tests was detected in two patients (cases no. 3 and 4). The decrease of AIRG to less than the first control percentile preceded impairment of oral glucose tolerance in three subjects who were tested for glucose tolerance at entry (cases no. 3 to 5).

Four ICA-positive individuals (cases no. 6 to 9), who previously displayed transient hyperglycemia and later developed type I diabetes, had low AIRG either at entry into the study or during follow-up evaluation (Table 1). None of the subjects at risk who remained normoresponders to glucose became insulin-dependent.

At entry, subjects who later progressed to diabetes (relatives and transiently hyperglycemic subjects) displayed lower mean AIRG, AIRGln, and AIRArg than ICA-positive nonprogressors ($P < .001$; Fig 3). However, AIRArg was affected less than AIRG and AIRGln in these subjects. Even a few months before diabetes onset, AIRArg was higher than AIRG and AIRGln ($P < .05$) and within the normal range, ie, all but one remained above the first control percentile and three of five were higher than the fifth control percentile.

The relation of overall serial AIRG, AIRGln, and AIRArg to the time before development of the disease in nine ICA-positive subjects who progressed to diabetes was studied using regression analysis (Fig 4). There was a progressive decline in AIRG and AIRGln ($P < .006, r = .5$ and .8, respectively) during the prediabetic phase. Conversely, there was no significant decrease in AIRArg with time.

To confirm the link between AIRs and worsening of prediabetes, the relation of AIRG, AIRGln, and AIRArg to basal glycemia was studied in ICA-positive progressors before the onset of diabetes, using regression analysis (Fig 5). AIRG progressively declined with increasing glycemia ($P < .05, r = .4$). Similarly, AIRGln tended to decrease, although not significantly, with increasing glycemia. Conversely, AIRArg was not influenced by basal glycemia, confirming the stability of AIRArg with time during the prediabetic phase (Fig 4).

To confirm the persistence of a substantial response to arginine, even at a late stage, responses to the different secretagogues were studied in a few diabetic patients either

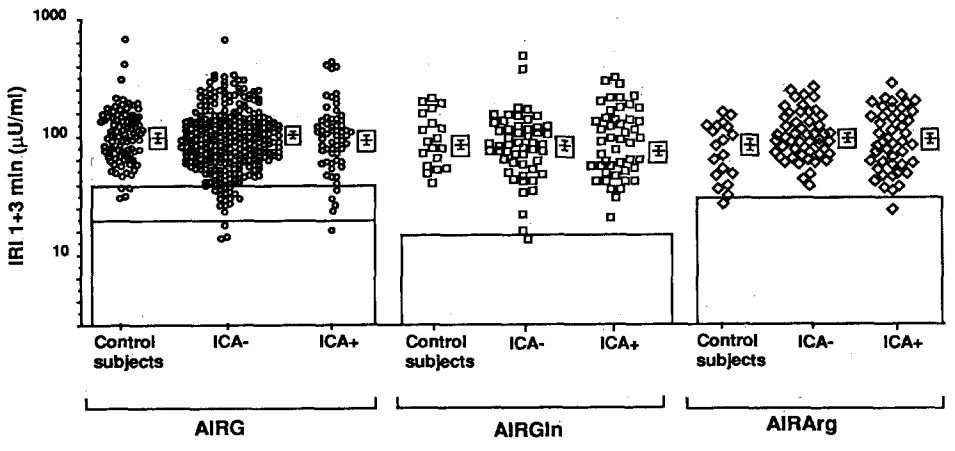

Fig 1 AIRG, AIRGln, and AIRArg expressed as IRI 1- + 3-minute values (μU/mL) in log scale; 89 healthy adult volunteers, 365 ICA-negative first-degree relatives of type I (insulin-dependent) diabetic patients, and 69 ICA-positive subjects at risk are shown. Vertical bars, mean ± SEM. Horizontal lines, 1st control percentiles for AIRG (20 μU/mL for prepubertal children and young adults, 37 μU/mL for Tanner's stages 2 to 5), AIRGln (15 μU/mL), and AIRArg (31 μU/mL).

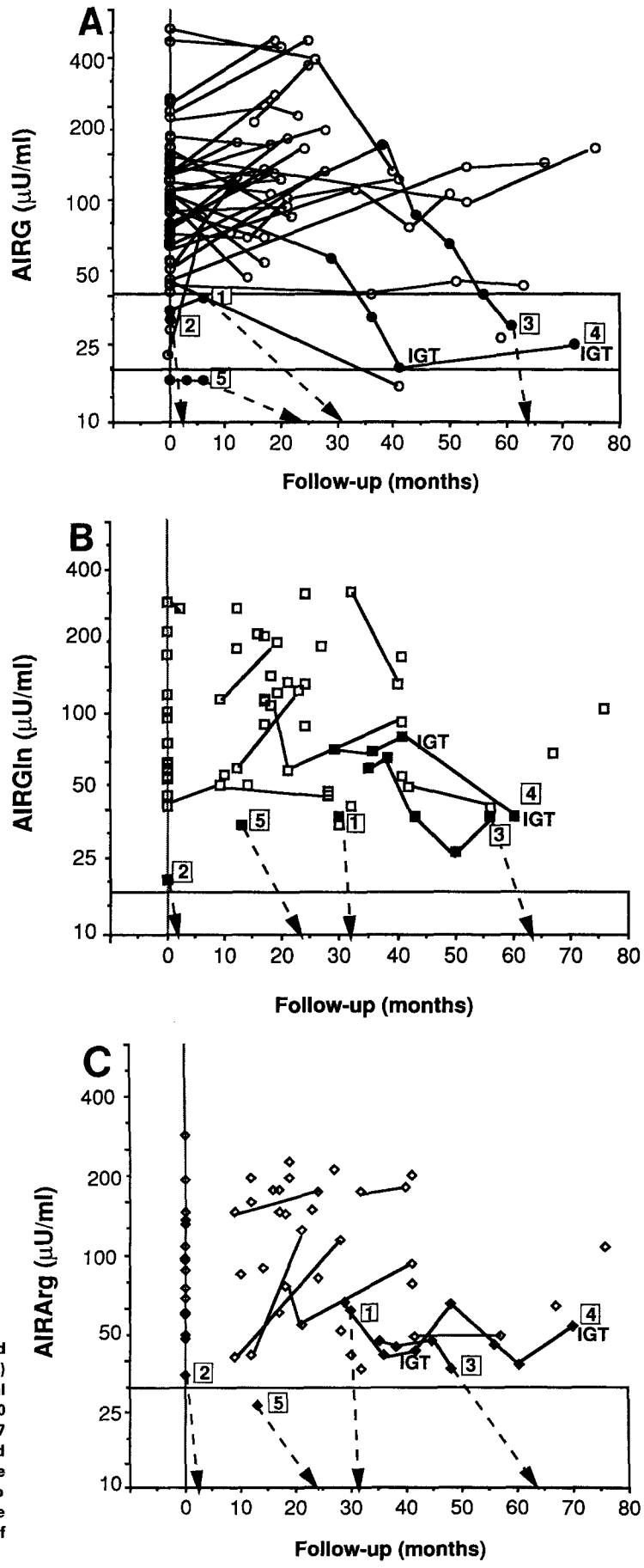

Fig 2. Follow-up study of AIRG (A), AIRGln (B), and AIRArg (C), expressed as IRI 1- + 3-minute values (μU/mL) in log scale in 69 ICA-positive subjects at risk. Horizontal lines indicate the 1st control percentiles for AIRG (20 μU/mL for prepubertal children and young adults and 37 μU/mL for Tanner's stages 2 to 5), AIRGln (15 μU/mL), and AIRArg (31 μU/mL). Sequential AIRs in each individual are linked by bond lines. (●, ■, ◆) Subjects who progressed to diabetes or IGT, represented by a number at the right of the last AIRG that refers to Table 1. (---) Delay to onset of diabetes.

Table 1. Characteristics of ICA-Positive Subjects at Risk Who Progressed to Type I Diabetes

Subject No.	Age at First Sample (yr)/Sex	Period From Diabetes (mo)	HLA-DR	ICA Human Pancreas	ICA Mouse Pancreas	GAD Antibodies (%)	64 KA	IAA (%)	AIRG* (μU/mL) 0	AIRG* (μU/mL) 1	AIRGln* (μU/mL) 0	AIRGln* (μU/mL) 1	AIRArg* (μU/mL) 0	AIRArg* (μU/mL) 1
Relatives														
1	14/M	30	3, 4	20	1:8	40	+	2.06	32	42	NT	34	NT	42
2	14/M	1	4	20	1:32	8.5	−	3.70	29	NT	25	NT	35	NT
3	11/M	63	4	160	1:32	14	+	1.50	68	30	59	37	47	46
4	13/F	48	3, 4	160	1:64	16	+	2.62	102	27	71	37	66	54
5	32/M	24	3, 4	5	1:4	6.1	+	0.71	18	17	NT	34	NT	27
Subjects with transient hyperglycemia														
6	26/F	6	3, 4	10	NT	NT	NT	0.40	16	NT	29	NT	22	NT
7	22/F	7	3, 4	160	NT	NT	NT	0.30	19	40	14	NT	NT	NT
8	29/F	14	4	20	NT	NT	NT	8.90	31	19	16	NT	49	NT
9	18/F	2	3	2.5	NT	NT	NT	NT	11	NT	38	NT	NT	NT

NOTE. Two results are given for metabolic tests: (0) results at entry into the study and (1) result at 6 months from diabetes. Titers of ICAs on human and mouse pancreases are given in JDF units and in last positive dilutions, respectively. For GAD antibodies, binding > 6.0% is considered positive. IAA values > 1.3% are considered positive.

Abbreviation: NT, not tested.

*One- and 3-minute insulin.

at onset of the disease or during non–insulin-receiving remission (Fig 4). AIRArg remained significant, ie, higher than the fifth control percentile, in five of nine patients, whereas AIRG was low, ie, less than the first percentile, in all but one subject. This pattern of partly preserved AIRArg during the remission phase is in agreement with previous studies.[42]

DISCUSSION

Low first-phase insulin response to IV glucose is an indicator of prediabetes and increases the predictive value of ICA positivity.[21,23,25] In this respect, all our subjects who later progressed to diabetes displayed this metabolic abnormality. However, it remains uncertain whether this parameter can contribute to determining the time of delay to disease. The initial hypothesis of a linear loss of β-cell response to glucose[21] has led to controversy. In our population of individuals who later progressed to diabetes, analysis of the relationship between overall serial AIRG and time before diabetes onset revealed a progressive decline of AIRG. However, the rate of decline varied among subjects, so that the absolute value of a single AIRG did not predict the delay time to diabetes. Moreover, for a given subject, even serial determinations of AIRG did not predict this delay, as illustrated by subject no. 4: although her AIRG declined linearly during 12 months preceding IGT, she is still free of diabetes 30 months after her first low AIRG.

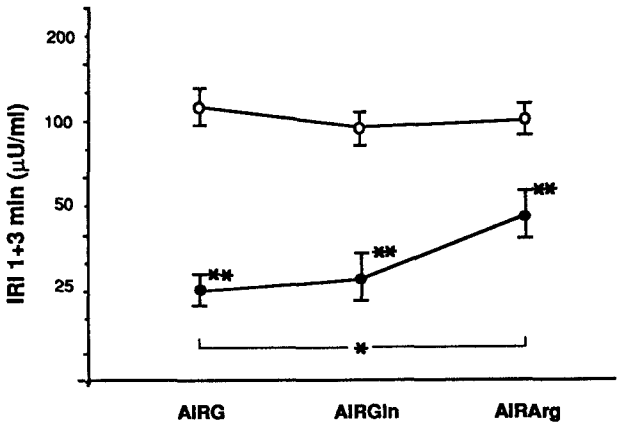

Fig 3. AIRG, AIRGln, and AIRArg, expressed as the mean ± SEM of IRI 1- + 3-minute values (μU/mL) in log scale in ICA-positive progressors (●) and ICA-positive nonprogressors (○) at entry to the study. Statistical differences are indicated between progressors and nonprogressors, and among progressors, between AIRG and AIRArg. *P < .05, **P < .001.

Our previous results from a cross-sectional study suggested that decreased AIR to nonglucose stimuli, especially arginine, may occur later than the loss of AIRG during pre–type I diabetes.[33] However, a prospective study was needed to confirm this hypothesis and to determine whether the occurrence of low AIRGln and AIRArg can predict the imminence of diabetes.

Each of three stimuli (glucose, glucagon, and arginine) had a relatively similar impact on the β-cell at entry into the present study, ie, the mean AIR for each stimulus and the spread of values were roughly the same. This was intentional, since we determined in a previous study[33] the injected amounts of glucose, glucagon, and arginine required to obtain maximal and similar responses for the three stimuli. This represents an advantage, since an identical initial level allows better evaluation over time of the differential decreases in AIR to each stimulus. Under these circumstances, the pattern in progression of AIRGln over time was similar to that of AIRG in prediabetic subjects. By contrast, although prediabetic subjects displayed lower mean AIRArgs than those who did not progress to diabetes, these responses were not totally blunted and overlapped with normal ranges. When the relationship of serial tests was analyzed with time before development of the disease or with increasing glycemia in subjects who progressed to diabetes, AIRArg appeared

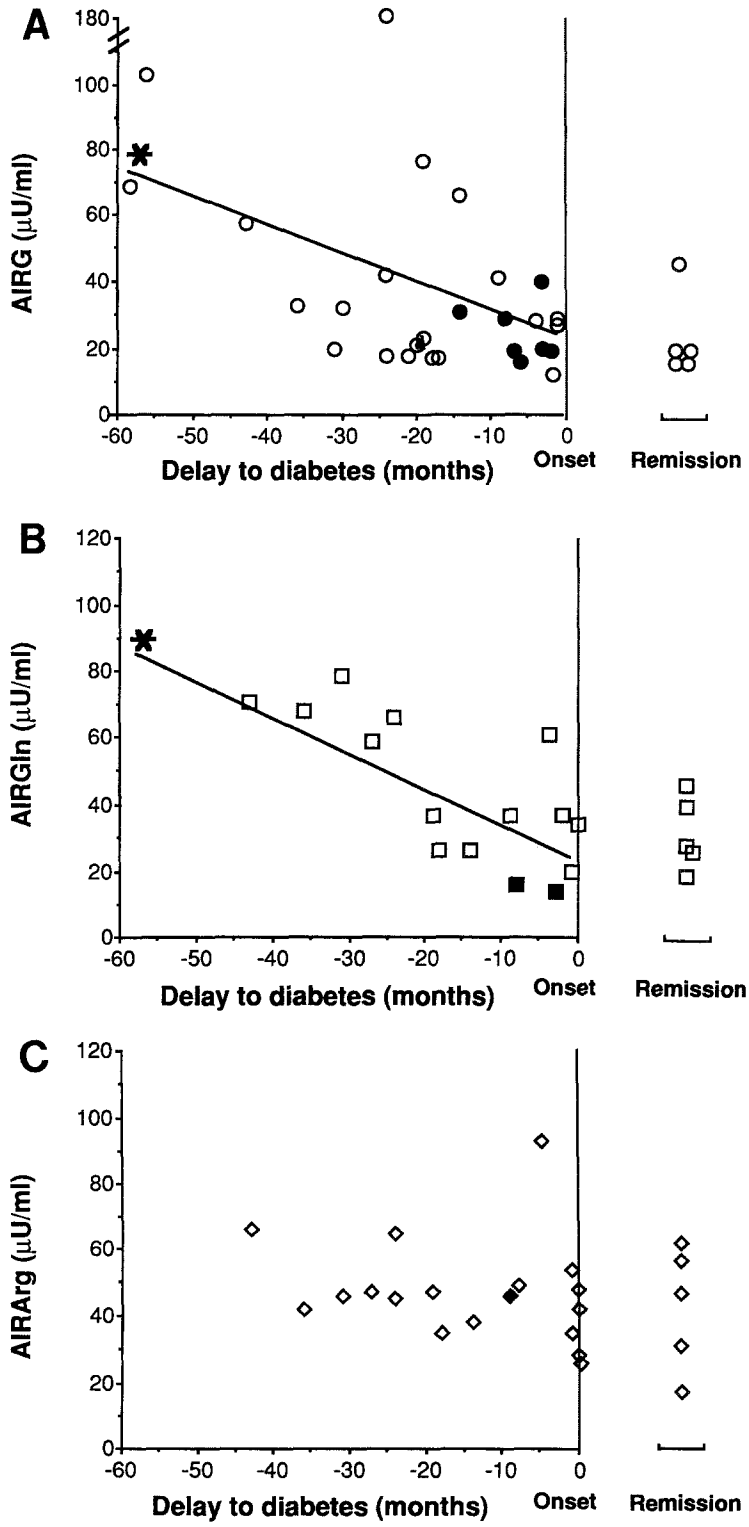

Fig 4. AIRG (A), AIRGln (B), and AIRArg (C) expressed as IRI 1- + 3-minute values in prediabetic subjects before onset of diabetes and in diabetic patients at onset or in remission. In prediabetic subjects, regression lines between delay to diabetes and both AIRG and AIRGln are significant ($y = -0.9x + 22$ and $y = -1.2x + 23$, respectively, $^*P < .006$). (●, ■, ◆) ICA-positive prediabetic subjects with previous transient hyperglycemia.

almost stable during the prediabetic phase, contrasting with the progressive decline in AIRG and AIRGln.

Moreover, in our study, patients with previous transient hyperglycemia were close to diabetes onset and displayed a less impaired AIRArg than AIRG, supporting the conclusion that even during late prediabetes, β-cell response to arginine is partly preserved and less altered than that to glucose. Such patterns of partly preserved AIRArg were also observed in recent-onset diabetic patients tested either at onset or during non–insulin-receiving remission.

Thus, on one hand, AIRArg and AIRGln proved to be of little use in improving disease prediction. However, even

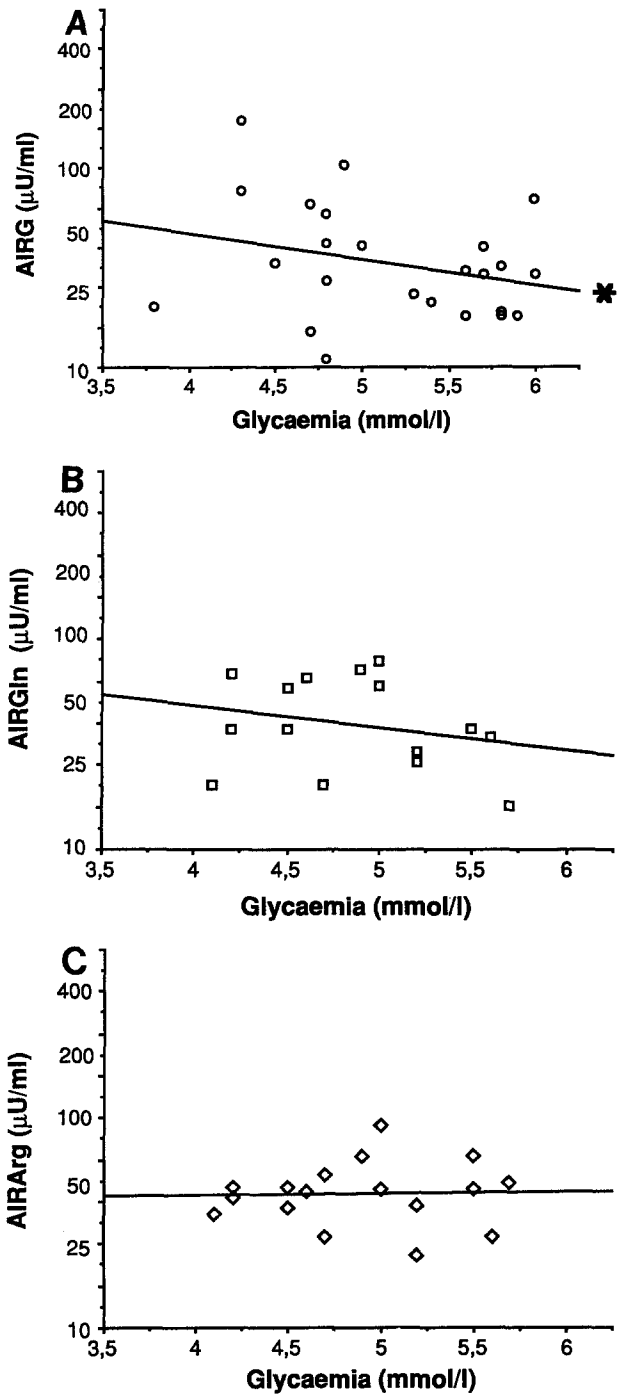

Fig 5. Relationship of AIRG (A), AIRGln (B), and AIRArg (C) expressed as IRI 1- + 3-minute values (μU/mL) in log scale with basal glycemia (mmol/L) in ICA-positive prediabetic subjects. Regression line between AIRG and glycemia is significant ($y = -0.2x + 2.4$, $*P < .05$).

though the study of differential responsiveness to glucose and arginine during the prediabetic phase seems of limited predictive value, it is of great interest to an understanding of the natural history of the β-cell defect during type I diabetes. Two factors may contribute to decreased insulin secretion. Decreased β-cell mass could explain why subjects who progress to diabetes display lower AIRs not only to glucose but also to other secretagogues, including arginine, than nonprogressors. Moreover, since glucose and arginine act differently on the β cell, a β-cell functional abnormality could account for differential responsiveness to glucose and arginine, with a more pronounced alteration in response to glucose. In this respect, even though sample size was small in the present study, it is noteworthy that basal insulin levels were low at onset of diabetes despite hyperglycemia, whereas AIRArg was partly preserved. A similar feature of differential AIRG and AIRArg, with more preserved AIRArg, was observed during remission phases. Thus, in addition to decreased β-cell mass, glucose-specific β-cell functional abnormalities may exist during prediabetes and even after the onset of clinical diabetes. Several hypotheses could explain such a functional abnormality. For example, it may be related to mechanisms revealed by antibodies directed against β-cell glucose transporters in humans[43] or in BB/W rats, in which a quantitative defect in GLUT2 transporters has been demonstrated.[44]

It would seem important to determine the respective influences of functional defects and decreased β-cell mass, which may differ from one subject to another. This is particularly relevant when preventive interventions are considered during prediabetes. Moreover, since β-cell functional abnormalities still appear to be a significant cause of decreased insulin secretion after clinical onset of diabetes, at least in some subjects, it can be supposed that the disease could still have some degree of reversibility even at this late stage, provided that functional abnormalities can be reversed.

ACKNOWLEDGMENT

The authors are grateful to the other members of the GOFEDI (Groupe Ouest-France pour l'Etude du Diabète Insulino-dépendant): A. Faivre (Service des Isotopes, Nantes); J.D. Bignon (Centre de Transfusion Sanguine, Nantes); S. Rémond (Pédiatrie, Nantes); J.M. Limal and M.G. Joseph (Pédiatrie, Angers); E. Mathieu, Y. Gallois, and A. Giraud (Laboratoire de Biochimie, Angers); H. Allannic (Endocrinologie, Rennes); M. Delamaire, M.C. Grais, M.C. Le Goff, G. Semana, and F. Vérité (Centre de Transfusion Sanguine, Rennes); C. Massart (Laboratoire d'Hormonologie, Rennes); L. Nduwayo (Endocrinologie, Tours); S. Fieuzal (Endocrinologie, Poitiers); and E. Praud (Pédiatrie, Le Mans).

REFERENCES

1. Castano L, Eisenbarth GS: Type 1 diabetes: A chronic autoimmune disease of man, mouse and rat. Annu Rev Immunol 8:647-679, 1990

2. Bonifacio E, Bingley PJ, Shattock M, et al: Quantification of islet-cell antibodies and prediction of insulin-dependent diabetes. Lancet 335:147-149, 1990

3. Tarn AC, Thomas JM, Dean BM, et al: Predicting insulin-dependent diabetes. Lancet 1:845-850, 1988

4. Riley WJ, Maclaren NK, Krischer J, et al: A prospective study of the development of diabetes in relatives of patients with insulin-dependent diabetes. N Engl J Med 323:1167-1172, 1990

5. Ziegler AG, Ziegler R, Vardi P, et al: Life-table analysis of progression to diabetes of antiinsulin autoantibody-positive rela-

tives of individuals with type 1 diabetes. Diabetes 38:1320-1325, 1989

6. Seißler J, Hering B, Richter W, et al: Antibodies to the Mr 64,000 (64K) protein in islet cell antibody positive non-diabetic individuals indicate high risk for impaired beta-cell function. Diabetologia 35:550-554, 1992

7. Atkinson MA, MacLaren NK, Scharp DW, et al: 64,000 Mr autoantibodies as predictor of insulin-dependent diabetes. Lancet 1:1357-1360, 1990

8. Bärmeier H, McCulloch DK, Neifing JL, et al: Risk for developing type 1 (insulin-dependent) diabetes mellitus and the presence of islet 64K antibodies. Diabetologia 34:727-733, 1991

9. Christie M, Landin-Olsson M, Sundkvist G, et al: Antibodies to a M_r-64000 islet cell protein in Swedish children with newly diagnosed type 1 (insulin-dependent) diabetes. Diabetologia 31:597-602, 1988

10. Baekkeskov S, Aanstoot HJ, Christgau S, et al: Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. Nature 347:151-156, 1990

11. Thivolet CH, Tappaz M, Durand A, et al: Glutamic acid decarboxylase (GAD) autoantibodies are additional predictive markers of type 1 (insulin-dependent) diabetes mellitus in high risk individuals. Diabetologia 35:570-576, 1992

12. Christie MR, Tun RYM, Lo SSS, et al: Antibodies to GAD and tryptic fragments of islet 64K antigen as distinct markers for development of IDDM. Studies with identical twins. Diabetes 41:782-787, 1992

13. Gianani R, Pugliese A, Bonner-Weir S, et al: Prognostically significant heterogeneity of cytoplasmic islet cell antibodies in relatives of patients with type 1 diabetes. Diabetes 41:347-353, 1992

14. Genovese S, Bonifacio E, Dean BM, et al: Distinct cytoplasmic islet cell antibodies with different risk of type 1 (insulin dependent) diabetes mellitus. Diabetologia 35:385-388, 1992

15. Timsit J, Caillat-Zucman S, Blondel H, et al: Islet cell antibody heterogeneity among type 1 (insulin-dependent) diabetic patients. Diabetologia 35:792-795, 1992

16. Saï P, Elmansour A, Audrain M, et al: Quantification of human cytoplasmic islet-cell antibodies which cross-react with mouse pancreas: A follow-up study in type 1 (insulin-dependent) diabetic patients and in first-degree relatives. Diabetologia 36:778-784, 1993

17. Chaillous L, Delamaire M, Martignat L, et al: Combined analysis of islet cell antibodies which cross-react with mouse pancreas, antibodies to the Mr 64,000 protein, and antibodies to intact glutamate decarboxylase, in type 1 diabetic patients. Diabetes Care 17:1115-1123, 1994

18. Chaillous L, Delamaire M, Maugendre D, et al: Islet cell antibodies which cross-react with mouse pancreas, antibodies to the Mr 64,000 protein, and antibodies to intact glutamate decarboxylase, in islet cell antibody positive subjects at risk of type 1 diabetes. Diabetologia 37:491-499, 1994

19. Bleich D, Jackson RA, Soeldner JS, et al: Analysis of metabolic progression to type 1 diabetes in ICA^+ relatives of patients with type 1 diabetes. Diabetes Care 13:111-118, 1990

20. Beer SF, Heaton DA, Alberti KGMM, et al: Impaired glucose tolerance precedes but does not predict insulin-dependent diabetes mellitus: A study in identical twins. Diabetologia 33:497-502, 1990

21. Srikanta S, Ganda OP, Gleason RE, et al: Pre type 1 diabetes: Linear loss of beta-cell response to intravenous glucose. Diabetes 33:717-720, 1984

22. Vardi P, Crisa L, Jackson RA: Predictive value of intravenous glucose tolerance test insulin secretion less than or greater than the first percentile in islet cell antibody positive relatives of type 1 (insulin-dependent) diabetic patients. Diabetologia 34:93-102, 1991

23. Vialettes B, Mattei-Zevaco C, Badier C, et al: Low acute insulin response to intravenous glucose. A sensitive but non specific marker of early stages of type 1 (insulin-dependent) diabetes. Diabetologia 31:592-596, 1988

24. Lo SSS, Hawa M, Beer SF, et al: Altered islet beta-cell function before the onset of type 1 (insulin-dependent) diabetes mellitus. Diabetologia 35:277-282, 1992

25. Bardet S, Joseph MG, Matthieu E, et al: Predictive value of age-related acute insulin response to glucose in subjects at risk for type 1 diabetes: Results of a 6-year follow-up study from west-France. Diabete Metab 39:372-380, 1993

26. McCulloch DK, Raghu PK, Johnston C, et al: Defects in β-cell function and insulin sensitivity in normoglycemic streptozotocin-treated baboons: A model of preclinical insulin-dependent diabetes. J Clin Endocrinol Metab 67:785-792, 1988

27. Bingley PJ, Matthews DR, Williams AJK, et al: Loss of regular oscillatory insulin secretion in islet cell antibody positive non-diabetic subjects. Diabetologia 35:32-38, 1992

28. Ganda OP, Srikanta S, Brink SJ, et al: Differential sensitivity to beta-cell secretagogues in "early" type 1 diabetes mellitus. Diabetes 33:516-521, 1984

29. Strandell E: Different sensitivity to glucose compared to other beta-cell secretagogues in female NOD mouse islets. Diabetologia 33:A95, 1990 (suppl, abstr)

30. Tominaga M, Komiya I, Johnson JH, et al: Loss of insulin response to glucose but not arginine during the development of autoimmune diabetes in BB/W rats: Relationships to islet volume and glucose transport rate. Proc Natl Acad Sci USA 83:9749-9753, 1986

31. Heaton DA, Lazarus NR, Pyke DA, et al: B-cell response to intravenous glucose and glucagon in non diabetic twins of patients with type 1 (insulin-dependent) diabetes mellitus. Diabetologia 32:814-817, 1989

32. Vialettes B, Zevaco-Mattei C, Thirion X, et al: Acute insulin response to glucose and glucagon in subjects at risk of developing type 1 diabetes. Diabetes Care 16:1-5, 1993

33. Bardet S, Rohmer V, Maugendre D, et al: Acute insulin response to intravenous glucose, glucagon and arginine in some subjects at risk for type 1 (insulin-dependent) diabetes mellitus. Diabetologia 34:648-654, 1991

34. Schmidli RS, Golman PG, Bonifacio E, et al: High level of concordance between assays for glutamic acid decarboxylase antibodies. The First International Glutamic Acid Decarboxylase Antibody Workshop. Diabetes 43:1005-1009, 1994

35. Snorgaard O, Hasselstrom K, Lumholtz IB, et al: Insulin/C-peptide response to intravenous glucagon. A dose-response study in normal and non-insulin dependent diabetic subjects. Acta Endocrinol (Copenh) 117:109-115, 1988

36. Van Haeften TW, Voetberg GA, Gerich JE, et al: Dose-response characteristics for arginine-stimulated insulin secretion in man and influence of hyperglycemia. J Clin Endocrinol Metab 69:1059-1064, 1989

37. Palmer JP, Benson JW, Walter RM, et al: Arginine-stimulated acute phase of insulin and glucagon secretion in diabetic subjects. J Clin Invest 58:565-570, 1976

38. Bardet S, Pasqual C, Maugendre D, et al: Inter and intra individual variability of acute insulin response during intravenous glucose tolerance tests. Diabete Metab 15:224-232, 1989

39. Gjessing HJ, Damsgaard EM, Matzen LE, et al: Reproducibility of β-cell function estimates in non–insulin-dependent diabetes mellitus. Diabetes Care 10:558-562, 1987

40. McCulloch DK, Klaff LJ, Kahn SE, et al: Nonprogression of

subclinical β-cell dysfunction among first-degree relatives of IDDM patients. 5-yr follow-up of the Seattle family study. Diabetes 39:549-556, 1990

41. Smith CP, Williams AJK, Thomas JM, et al: The pattern of basal and stimulated insulin responses to intravenous glucose in first-degree relatives of type 1 (insulin-dependent) diabetic children and unrelated adults aged 5 to 50 years. Diabetologia 31:430-434, 1990

42. Dupre J, Jenner MR, Mahon JL, et al: Endocrine-metabolic function in remission-phase IDDM during administration of cyclosporine. Diabetes 40:598-604, 1991

43. Johnson JH, Crider BP, McCorkle K, et al: Inhibition of glucose transport into rat islet cells by immunoglobulins from patients with new-onset insulin-dependent diabetes mellitus. N Engl J Med 322:653-659, 1990

44. Orci L, Unger RH, Ravazzola M, et al: Reduced beta-cell glucose transporter in new onset diabetic BB rats. J Clin Invest 86:1615-1622, 1990