Association of IDDM and Attenuated Response of 2′,5′-Oligoadenylate Synthetase to Yellow Fever Vaccine

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Basal and yellow fever vaccination–induced 2′,5′-oligoadenylate synthetase (2′,5′A) activity was determined in blood mononuclear cells (peripheral blood lymphocytes [PBLs]) from insulin-dependent diabetes mellitus (IDDM) and matched control subjects. The live attenuated yellow fever vaccine represented a primary stimulus in all subjects. First, basal 2′,5′A activity increased severalfold in response to yellow fever vaccination. In IDDM subjects, this increase was significantly lower (P = .025). Second, the 2′,5′A activity increased proportionately to the higher basal 2′,5′A activity in IDDM subjects. In control subjects, the increase in 2′,5′A activity was not dependent on the basal activity. There was no relationship between basal or stimulated 2′,5′A activity and age, sex, duration of IDDM, age at onset of IDDM, metabolic control, or HLA-DQ β-chain gene polymorphism. There is a direct relationship between 2′,5′A activity and latent viral infections associated with the presence of double-stranded RNA and with cellular interferons (IFNs) formed in response to viral infections. The higher basal 2′,5′A activity (P = .05) in relation to the stimulated activity may therefore signify a latent infection or the presence of double-stranded RNA in PBLs of IDDM subjects. In vitro stimulation of PBLs showed increased IFN sensitivity in IDDM subjects. Analysis of 2′,5′A activity is proposed to be a sensitive measure of the activation of the IFN system and the level of latent infectivity. *Diabetes* 38:1636–42, 1989

In immune reactions against viruses, there is a marked increase in 2′,5′-oligoadenylate synthetase (2′,5′A). This enzyme is induced by all three interferons (IFN-α, -β, and -γ) to different degrees (1–3) but is also highly sensitive to double-stranded RNA (dsRNA) (4–10). The enzyme is activated in T- and B-monocytes and natural killer (NK) cells and produces oligoadenylates from cellular ATP. Furthermore, the IFN effect involves the activation of a latent RNase and a phosphodiesterase leading to inhibition of protein synthesis and increased cleavage of RNA in somatic cells infected or uninfected by viruses (11–13).

The production of class II molecules as a restriction element for presentation of virus antigen is thought to be responsible for the initiation of virus elimination (14–21). In this connection, IFN-γ increases gene expression of HLA class I and II antigens, and both IFN-α and IFN-β stimulate class I expression (17–21). Increased class II molecule expression may also be involved in autoimmune processes (22–25). If HLA antigens are aberrantly expressed by exposure to viral infections, a persistent viral infection (26), which can inhibit the 2′,5′A activity (27), might eventually develop.

We tested whether there is a relation between the IFN system and the 2′,5′A system as a pathogenic element in insulin-dependent diabetes mellitus (IDDM). This was considered reasonable because there is evidence for 2′,5′A gene polymorphism (28,29), the 2′,5′A gene is the major gene that is induced after IFN stimulation of cells (1), and 2′,5′A genes and class I and II genes share regulatory sequences thought to be controlled by IFN (28). Because it has been found that pancreatic islet cells exposed to IFN in vitro show increased expression of class I and II antigens and immunoreactive IFN-α have been located at the β-cells in IDDM (30,31), a latent viral infection may reflect the pathogenesis of islet β-cell destruction. By use of a controlled in vivo immunization with yellow fever vaccine, it was possible to relate basal to stimulated 2′,5′A activity and draw comparisons between a healthy control group and an IDDM group. We also performed an analysis of HLA-DQ β-chain gene polymorphism to exclude the possibility that the dif-

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ferences observed were related to this IDDM-susceptibility marker (32).

RESEARCH DESIGN AND METHODS

Chemicals. Poly(I):poly(C) and ATP were obtained from P-L Biochemicals (Pharmacia, Uppsala, Sweden); acid alumina WA-1, bovine serum albumin (BSA), and alkaline phosphatase were obtained from Sigma (St. Louis, MO); and columns for ion-exchange chromatography were obtained from Bio-Rad (Richmond, CA). Creatine kinase and creatine phosphate were obtained from Boehringer Mannheim (Mannheim, FRG), Nonidet P-40 was obtained from Calbiochem-Behring (La Jolla, CA) and radiolabeled [α-^{32}P]ATP was obtained from Amersham (Buckinghamshire, UK). IFN-α was generously supplied by Hoffmann-LaRoche (Basel).

Immunization of subjects. Ten women and 23 men with IDDM (median age 36 yr) and 10 nondiabetic women and 21 nondiabetic men (median age 35 ± 5 yr) participated in the study after informed consent was obtained. The diabetic subjects were from our outpatient clinic, and their degree of metabolic control was judged by HbA_{1c} measurements (Table 1). None of the participants had been to an area where yellow fever is endemic. The study was performed in agreement with the Helsinki II declaration and was approved by the regional ethical committee. Attenuated yellow fever virus vaccine (Arilvax, 17D strain, batch BY F/5/126, Wellcome, Beckenham, UK) was used for immunization.

Lymphocyte preparation. Heparinized blood was drawn before the yellow fever vaccination on days 1 and 8. Two milliliters heparinized blood was layered on top of 3 ml Ficoll-Hypaque and centrifuged (800 × *g*) at room temperature for 20 min. The interphase with mononuclear cells was transferred to conical tubes and washed three times with Hanks' buffer containing 1% BSA (pH 7.4). The cells were counted and adjusted to 10^6/ml. Viability was always >98%. Cell lysis was achieved with 0.5% (vol/vol) Nonidet P-40, and homogenates were stored at −80°C. Protein determination was done with the BCA protein reagent (Pierce, Rockford, IL).

Analysis of 2′,5′A. This was done as previously described (9). In brief, 10 μl Nonidet P-40 homogenate corresponding to 10^5 mononuclear cells was incubated at 37°C for 105 min in 20 mM HEPES (pH 8) containing 2.5 mM ATP, 20 mM magnesium acetate, 10 mM creatine phosphate, 3 mg/ml creatine kinase, 10% glycerol, 20 μg/ml poly(I):poly(C), and 1.66–3.32 kBq [α-^{32}P]ATP/tube. Radioactive phosphorus not used in the diester bonds of the synthesized 2′,5′-oligoadenylates was removed with bacterial alkaline phosphatase. Separation of the oligoadenylates from free [α-^{32}P]ATP was achieved with a 400-μl acid alumina column washed with a 1 M glycine-HCl buffer (pH 2). 2′,5′A activity was expressed in units per milligram protein or as percent change, with units defined as nanomoles ATP converted per minute. Elution was done with 3 ml of the glycine buffer eluted directly to plastic scintillation vials and counted in the ^{3}H channel by Cerenkov radiation in a Packard (Tricarb) scintillation counter.

Incubation studies. Before and after yellow fever immunization, isolated peripheral blood lymphocytes (PBLs; 10^6 cells/ml) were incubated at 37°C for 18 h in RPMI-1640 with 10% fetal calf serum in a 95% O_2/5% CO_2 atmosphere. IFN-α was dissolved in RPMI-1640 buffer and added in increasing concentrations as indicated. Incubations were terminated by repeated washings in RPMI-1640 buffer, and cellular lysate was prepared as described above.

Leukocyte count, Igs, C-reactive protein (CRP), and HbA_{1c}. Leukocytes were counted with a Coulter S counter (Luton, UK), whereas a differential count was performed manually. Igs were determined by conventional immunoprecipitation on a Cobas Fara centrifugal analyzer (Roche, Basel), and CRP was determined by rocket immunoelectrophoresis (33). HbA_{1c} analysis was performed by high-performance liquid chromatography.

Yellow fever antibodies. IgG antibodies against yellow fever and plaque-reduction neutralization tests were performed by T. Monath (Dept. of Health and Human Services, Fort Collins, CO). IgG antibodies were determined by conventional enzyme-linked immunosorbent assay. Seroconversion, as determined by plaque-reduction neutralization test, was positive at the highest serum dilution that would give a viral plaque reduction ≥90%.

Restriction-fragment–length polymorphism (RFLP). DNA preparation, genomic blotting, and hybridization were done as previously described except that nitrocellulose membranes were used instead of nylon filters (27).

Statistical methods. Testing for differences was done by Mann-Whitney *U* test or Spearman's rank-correlation coefficient when appropriate. Basal and stimulated levels of 2′,5′A were log-normal distributed. Log-transformed data were tested with Student's *t* test. Further analysis included regression analysis and calculation or tolerance ellipses (34).

TABLE 1
Clinical data, differential count, and 2′,5′-oligoadenylate synthetase (2′,5′A) activity in insulin-dependent diabetes mellitus (IDDM) and control subjects

	IDDM	Control
Mean age (yr)	35.5 (18–49)	36 (20–35)
n (F/M)	10/23	10/21
Duration of IDDM (yr)	13.5 (2–33)	
Insulin dose (IU/24 h)	44 (25–74)	
Differential count		
Leukocytes (10^3/ml)		
Day 1	6.90 ± 2.71	5.71 ± 0.92
Day 8	5.32 ± 2.50	4.67 ± 1.12
Polymorphonuclear cells (%)		
Day 1	61 ± 10	58 ± 9
Day 8	56 ± 12	53 ± 9
Lymphocytes (%)		
Day 1	20 ± 10	33 ± 8
Day 8	31 ± 10	32 ± 8
Monocytes (%)		
Day 1	6 ± 3	6 ± 2
Day 8	8 ± 3	10 ± 4
2′,5′A activity in PBLs (U/mg protein)		
Day 1	1.53 ± 0.98	1.04 ± 0.50
Day 8	8.92 ± 7.71	7.69 ± 4.20

PBLs, peripheral blood lymphocytes. Values in parentheses indicate range. Sampling was done before (day 1) and after (day 8) vaccination with yellow fever.

RESULTS

Yellow fever vaccination. To exclude the possibility of a different time course in 2',5'A activity in IDDM versus control subjects, three IDDM and six control subjects were examined on days 1–22. We observed no differences in time needed to reach maximum 2',5'A activity or in half times after the maximum enzyme activity.

Only negligible subjective discomfort was experienced by the subjects after the yellow fever vaccination. There was a minor increase in PBLs on day 8 after vaccination both in IDDM and control subjects. However, the increase was not significant, and there was no difference between the two groups. Levels of CRP and IgA, IgG, and IgM were unaffected by vaccination and IDDM.

Yellow fever antibodies and neutralization test. Yellow fever IgG antibodies were below the detection limit of the assay in both IDDM and control subjects on days 1 and 8. This was also the case in the plaque-reduction neutralization test. Time-course studies after a primary vaccination showed that the neutralization test did not show any significant increase in titers until after day 10 (unpublished observation).

2',5'A activity pre- and postimmunization. There was no relationship between 2',5'A activity, age of the participants, sex, age at onset, or duration of diabetes. The level of 2',5'A activity in PBLs before and after vaccination is shown in Table 1. The percent increase in 2',5'A activity from day 1 to day 8 was 489 ± 284% in IDDM subjects and 753 ± 491% in control subjects ($P < .025$; Table 1). Stimulated and basal levels of 2',5'A activity were log-normal distributed in both populations (Fig. 1). The mean basal level of 2',5'A activity was higher in IDDM subjects ($P = .05$; Table 1).

The effect of vaccination was evaluated by regression analysis and construction of tolerance ellipses (Fig 2). Using an equation plot, we took into account the individual basal 2',5'A levels on day 1. Regression equations for the values are shown in the legend to Fig. 1. The two groups' reactions to immunization were highly different, with IDDM subjects reacting proportionately to their basal level of 2',5'A activity (resulting in a horizontal regression line in Fig. 2; deviation from 0 NS; $r = -.1021$). This is in sharp contrast to the control subjects, in whom the 2',5'A activity increased with a nearly constant value regardless of the basal level. This resulted in a negative regression line in Fig. 2 ($r = .5197$, $P < .01$), denoting a statistically significant difference between the two populations.

Metabolic control and 2',5'A activity. To evaluate the question of metabolic influence on 2',5'A activity, the relationship between HbA_{1c} and the increase in 2',5'A activity was examined (Fig. 3). There was no decrease ($r = -.2706$, $P = .13$) in the percent increase in 2',5'A activity with increasing HbA_{1c} level. Similarly, there was no significant relationship between HbA_{1c} and basal 2',5'A activity ($r = -.1644$, $P = .36$).

Stimulation in vitro with IFN-α. Before vaccination (day 1) and on day 8, PBLs were stimulated in vitro with human recombinant IFN-α in increasing concentrations (Fig. 4). Sensitivity to IFN-α was defined as the IFN concentration needed to elicit half-maximal response in 2',5'A activity. In IDDM subjects, the IFN-α concentration was 36 ± 17 U/ml, and in control subjects the concentration was 56 ± 11 U/ml ($P = .01$). In the vaccinated state, i.e., on day 8 when the maximal in vivo 2',5'A activity was observed, no differences were found (data not shown).

RFLP and 2',5'A activity in IDDM and control subjects. Analysis of DNA with the HLA-DQβ first-intervening–sequence probe detecting *Bam*HI 12– and 4–kilobase pair (kb) fragments revealed no relationship between the HLA-DQ gene polymorphism, basal 2',5'A activity, or stimulated reac-

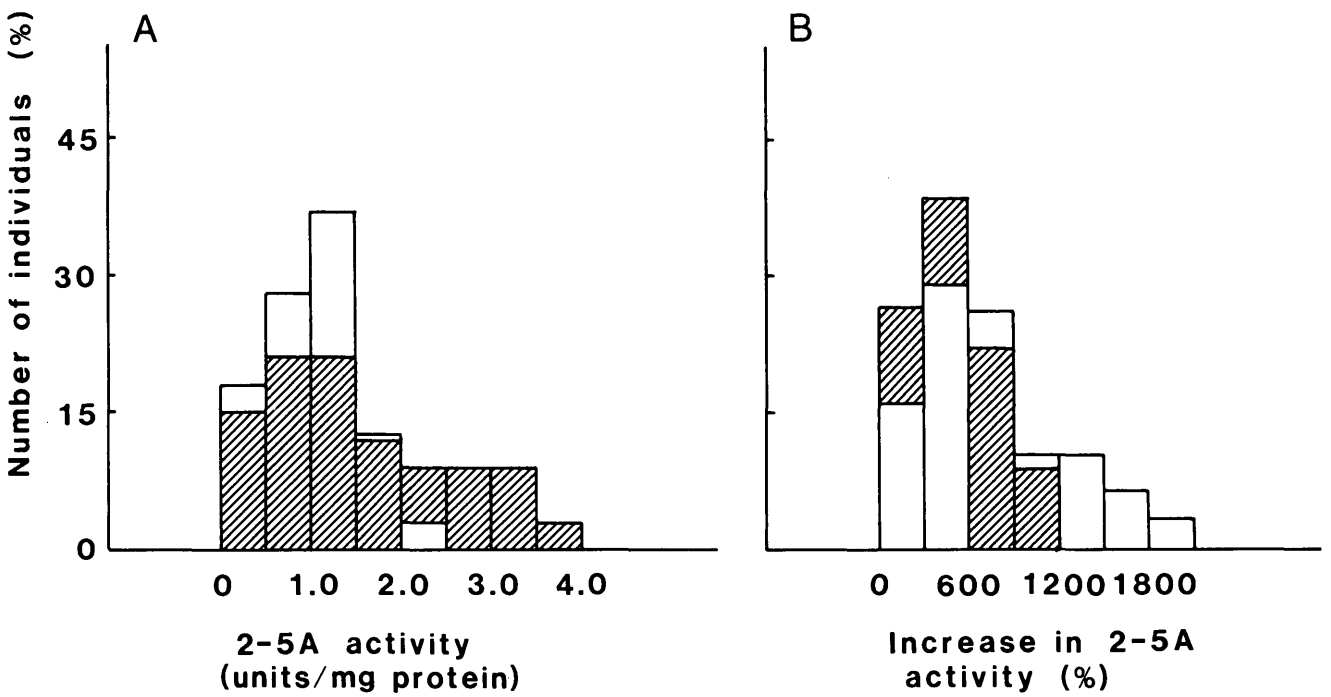

FIG. 1. *A*: distribution of basal 2',5'-oligoadenylate synthetase (2-5A) activity in Ficoll-Hypaque–isolated control (*open bars*) and insulin-dependent diabetes mellitus (*hatched bars*) peripheral blood lymphocytes on day 1. *B*: distribution of 2-5A activity increases on day 8.

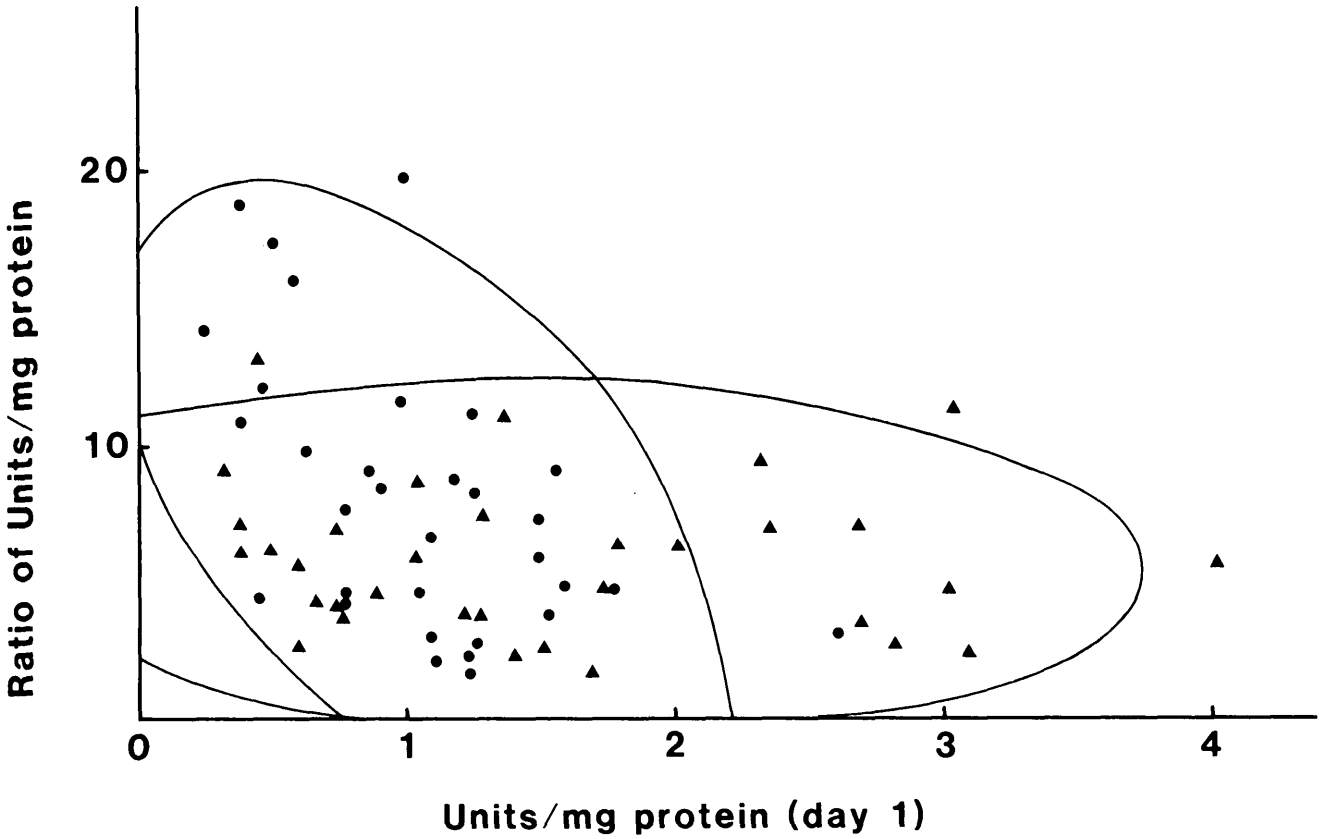

FIG. 2. 2′,5′-Oligoadenylate synthetase activity in peripheral blood lymphocytes on day 1 in relation to day 8/day 1 ratio of activity. Tolerance ellipses comprising 90% of two populations were constructed. Regression line is expressed as $y = -5.2938x + 13.7616$ ($r = -.5197$, $P < .01$) for control subjects (●) and $y = -0.2955x + 6.4515$ ($r = -.1021$, NS) for insulin-dependent diabetic subjects (▲).

tivity (i.e., percent increase from days 1 to 8). This was the case for control and IDDM subjects (Table 2). Independent of RFLPs, control subjects had a higher increase in 2′,5′A, whereas IDDM subjects had a higher basal level of 2′,5′A.

DISCUSSION

In this study, we compared the IFN-dependent 2′,5′A activity in isolated PBLs from nondiabetic and IDDM subjects before and after immunization with yellow fever vaccine. In a previous study (9), a marked increase in 2′,5′A activity was observed on day 9 when nondiabetic subjects were vaccinated with the live attenuated yellow fever vaccine. In this study, we compared the IFN-dependent 2′,5′A activity in isolated PBLs from nondiabetic and IDDM subjects before and after immunization with yellow fever vaccine. It was demonstrated that after yellow fever vaccination, a significantly smaller increase in 2′,5′A activity occurred in IDDM subjects. Surprisingly, however, this increase was proportional to the basal level. Thus, in IDDM subjects, the higher the basal 2′,5′A activity in PBLs the higher the stimulated activity. Together with the smaller vaccinatory 2′,5′A increase, however, this would signify attenuated 2′,5′A activity after yellow fever.

We investigated whether there would be any differences in the primary immune response (expressed by IFN-dependent 2′,5′A activity) when nondiabetic and IDDM subjects were exposed to a live attenuated virus. This seems relevant for several reasons. First, the IFN-dependent 2′,5′A enzyme is probably the most important enzyme in the process of inhibiting and controlling viral infections (35–37). Second, the IFNs also have an effect on HLA class II–antigen expression, which may play an important pathogenic role in eliciting and perpetuating autoimmune mechanisms (22,23). Finally, considering the possibility of a latent viral infection (26,27) or the presence of other genomic sequences in humans (38), these factors most likely would influence the activity level of 2′,5′A. Thus, the 2′,5′A system could be used as a marker for IFN activity or silent viral infections.

We propose herein that a persistent viral infection or presence of foreign genomic material could lead to our observations (27,38). A latent infection would tend to increase basal 2′,5′A activity, resulting in a reduced 2′,5′A response to a challenging virus (e.g., yellow fever). Also, because of the sensitivity of 2′,5′A to dsRNA, the presence of such nucleotide sequences would provide increased amounts of substrate for the enzyme (39). These possibilities would lead to higher basal enzyme activity in IDDM subjects and the observed proportional 2′,5′A increase simultaneous with reduced maximal response.

The differential count of PBLs did not reveal any differences between IDDM and control subjects. However, it cannot be excluded that a different distribution of PBLs in the two populations would influence our results. In separate experiments, however, we found no evidence of different subpopulations of lymphocytes (i.e., β-cells, CD4, CD8, natural killer cells, and monocytes; unpublished observations). On the other hand, increases in 2′,5′A after exposure to IFN-α

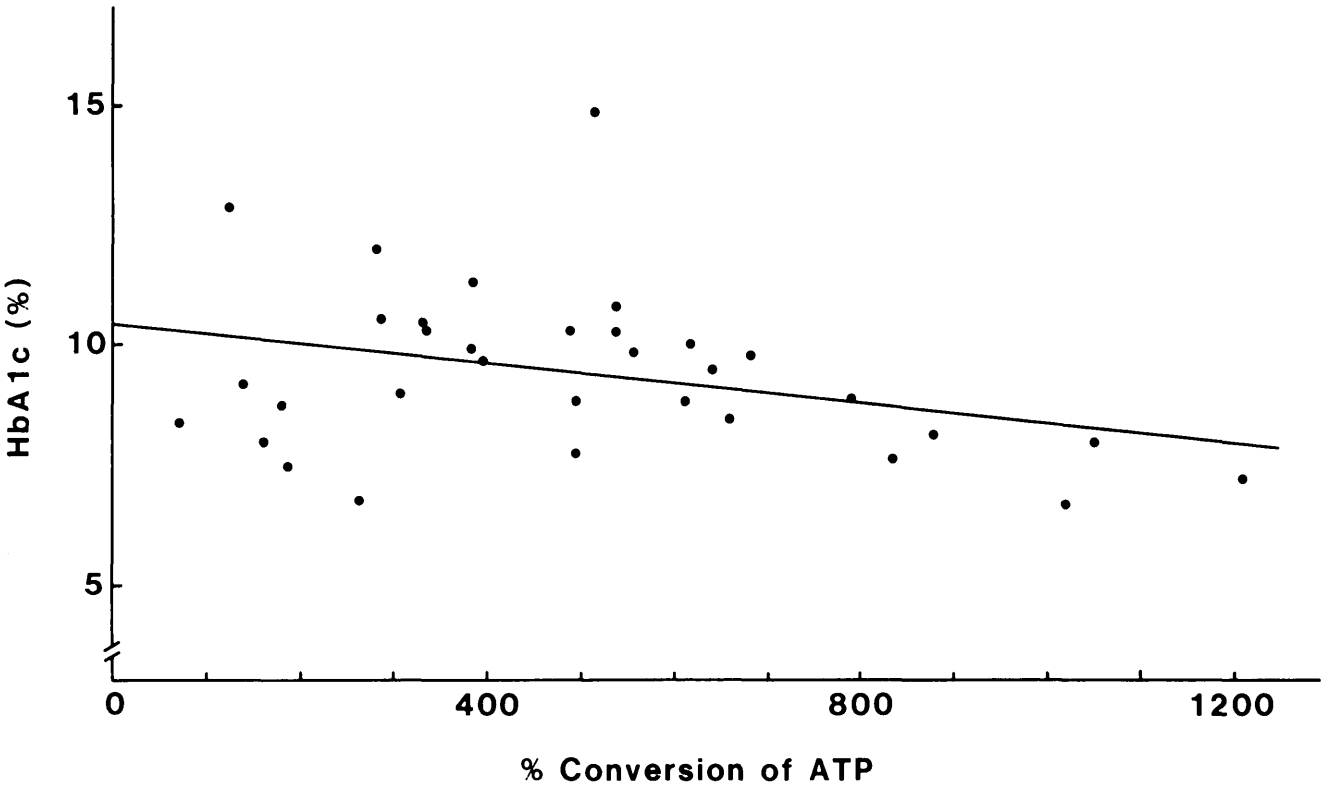

FIG. 3. 2',5'-Oligoadenylate synthetase activity increase in insulin-dependent diabetic subjects in relation to HbA_{1c}. Regression line is expressed as $y = -0.002x + 10.43$ ($r = -.2706$, NS).

were comparable in PBL subsets (11). Further studies will be needed on this question; it is still debatable whether PBL subsets in IDDM subjects differ from those in control subjects (40).

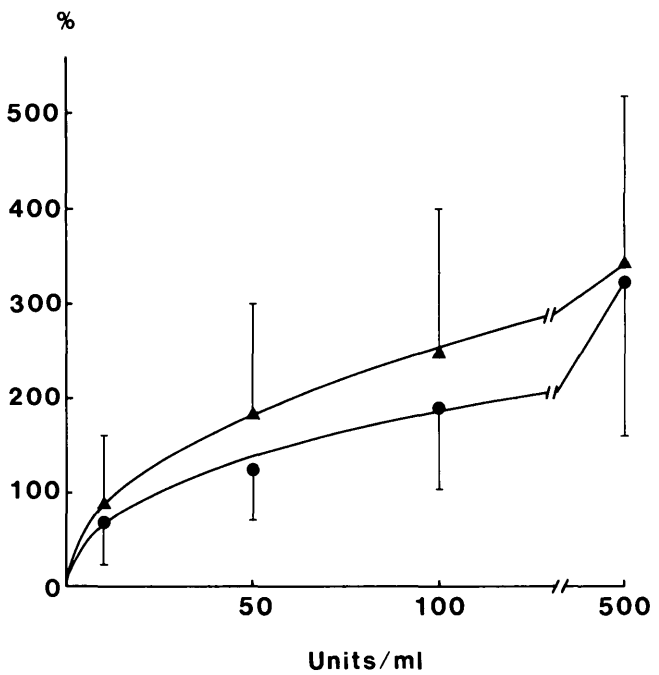

FIG. 4. In vitro effects of interferon (IFN-α) on 2',5'-oligoadenylate synthetase (2',5'A) activity. Ficoll-Hypaque–isolated peripheral blood lymphocytes (10^6/ml) were incubated for 18 h in presence of recombinant human IFN-α (0–500 U/ml). 2',5'A activity for insulin-dependent diabetes mellitus (▲, $n = 32$) and control (●, $n = 29$) subjects are expressed as increase (%) in 2',5'A activity at 0 concentration. Values are means ± SD.

The in vivo differences observed between IDDM and control subjects seem to find support in our in vitro data. The half-maximal 2',5'A response occurred at a much lower IFN-α concentration in IDDM subjects, indicative of an increased sensitivity to IFN-α.

However, the maximal response to IFN-α did not differ, indicating a similar receptor number per cell in the two groups. The increased sensitivity in vivo should then be caused by increased receptor affinity and not solely by increased receptor number. This would not fit with our data, because increased receptor binding would elicit down-regulation of specific receptors. We found no evidence of this because the in vivo basal 2',5'A activity in IDDM subjects was higher and sensitivity to IFN-α in vitro was higher. Therefore, we suggest that the 2',5'A activity in IDDM subjects is the result of specific IFN-α binding (41) with a primed 2',5'A enzyme as discussed for the in vivo findings.

The association between IDDM and the HLA-DQβ first-intervening–sequence probe is evidenced from a high prevalence of 12- and 4-kb fragments in IDDM (32). As demonstrated, we found no connection between the HLA-DQ gene and 2',5'A activity. Even if the frequencies of the different restriction fragments in IDDM and control subjects are comparable to our previous findings (32), the differences in 2',5'A between IDDM and control subjects cannot be explained by differences in HLA-DQ. More IDDM and control subjects will be needed to evaluate a possible relation between HLA-DQ and 2',5'A expression. It was found that the 2',5'A gene maps to chromosome 12 (42). The complicated relationships show that *1*) IFN increases transcription of MHC genes (43), *2*) there are IFN-responsive regulatory elements in the promoter region of the 2',5'A gene (44), and 3) regulatory-gene sequences are shared between MHC and

TABLE 2
Basal and stimulated 2′,5′-oligoadenylate synthetase (2′,5′A) activity in relation to restriction fragments after *Bam*HI digestion and hybridization with the HLA-DQIVS1 probe in insulin-dependent diabetes mellitus (IDDM) and control subjects

	2′,5′A activity					
	Control (n = 29)			IDDM (n = 30)		
HLA-DQIVS1 fragments (kb)	n	Basal (U/mg)	Day 1 (%)	n	Basal (U/mg)	Day 1 (%)
12–4	2 (7)	0.94	804	9 (30)	1.87 ± 1.02	526 ± 285
12 and/or 4	22 (76)	1.33 ± 1.08	697 ± 484	24 (80)	1.54 ± 1.00	484 ± 251
Non-12/non-4	7 (24)	0.97 ± 0.43	1000 ± 445	3 (10)	2.13 ± 1.00	688 ± 257
7.5–3	4 (14)	1.06 ± 0.38	987 ± 416	2 (7)	2.03	594

The 2′,5′A activity was measured in Ficoll-Hypaque–isolated peripheral blood lymphocytes. No statistically significant differences or connections between IDDM or control subjects and 2′,5′A activity were detected. Values in parentheses are percentages. Due to lack of material, 2 subjects are missing in the control group and 3 in the IDDM group. kb, Kilobase pairs.

2′,5′A gene sequences (28). Further research will clarify what role these factors are playing, alone or in concert, for the changed 2′,5′A activity in IDDM subjects.

Summarizing, we found attenuated 2′,5′A activity in IDDM subjects after exposure to yellow fever vaccine. The attenuated response was due to a higher basal 2′,5′A activity before vaccination. Besides, the 2′,5′A activity increased proportionately to the basal activity. These changes may be caused by the presence of a latent viral infection.

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