

SUPPLEMENTARY DATA

Supplementary Table 1. Baseline characteristics, according to study group.

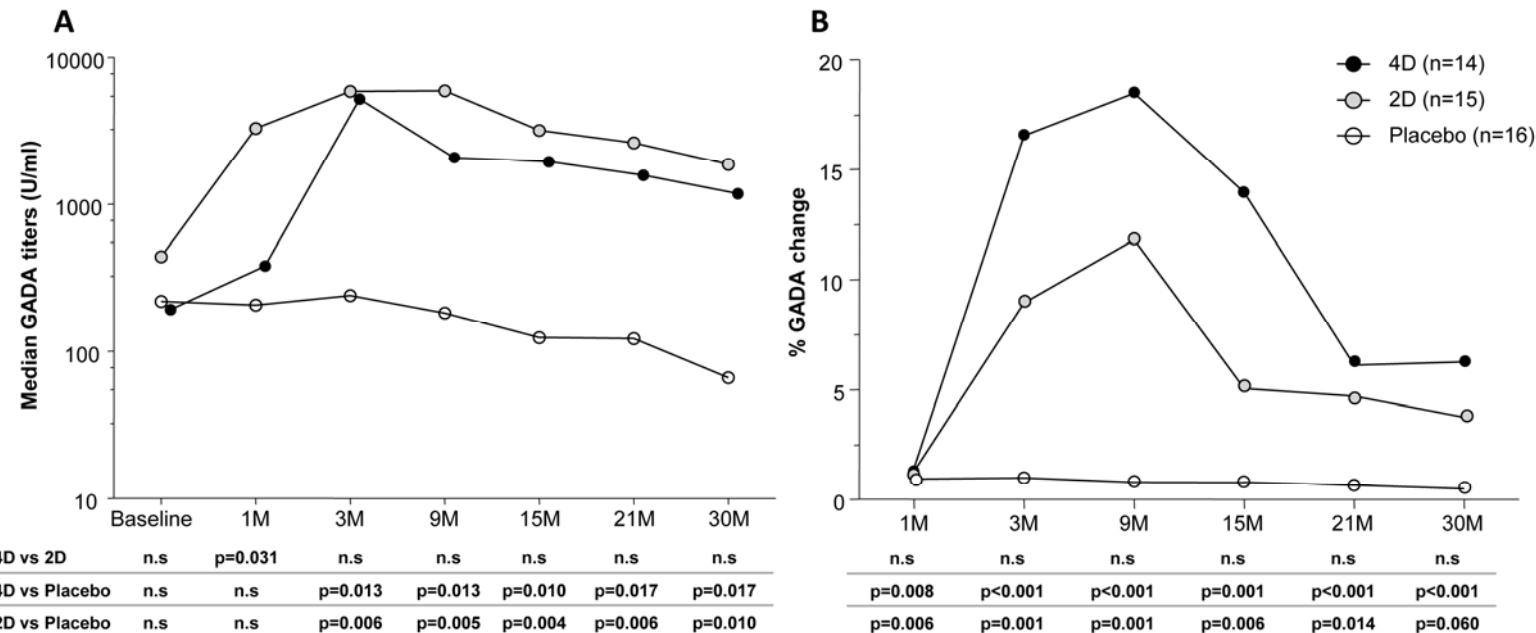
Characteristic	Swedish subgroup			Entire Swedish cohort			Entire study cohort		
	4D (n=14)	2D (n=15)	placebo (n=16)	4D (n=49)	2D (n=49)	placebo (n=50)	4D (n=111)	2D (n=108)	placebo (n=115)
Age, years	13.2±2.4	13.3±2.4	13.4±2.7	13.3±2.1	13.3±2.2	13.4±2.5	12.9±2.4	12.9±2.1	13.3±2.3
Days since diagnosis	76.4±26.1	67.7±22.8	67.6±23.5	74.6±20.3	73.3±20.0	71.0±20.6	73.6±25.0	76.9±23.3	74.2±23.4
Gender distribution, n (%)									
Female	9 (64)	6 (40)	8 (50)	25 (51)	26 (53)	20 (40)	58 (52)	48 (44)	55 (48)
Male	5 (36)	9 (60)	8 (50)	24 (49)	23 (47)	30 (60)	53 (48)	60 (56)	60 (52)
HLA classification, n (%)									
Very high risk	3 (21.3)	7 (47)	6 (38)	14 (29)	18 (37)	16 (32)	30 (28)	30 (28.3)	31 (28.2)
High risk	6 (43)	6 (40)	5 (31)	21 (44)	23 (47)	23 (46)	41 (38.3)	41 (38.7)	40 (36.4)
Moderate risk	3 (21.3)	2 (13)	4 (25)	8 (17)	4 (8)	8 (16)	24 (22.4)	23 (21.7)	28 (25.5)
Low risk	2 (14.3)	0 (0)	1 (6)	5 (10)	4 (8)	3 (6)	12 (11.2)	12 (11.3)	11 (10)
<i>Total Observations</i>	14	15	16	48	49	50	107	106	110
Tanner, n (%)									
1	2 (14.3)	4 (27)	0 (0)	6 (12)	10 (20)	5 (10)	15 (14)	22 (21)	12 (11)
2+3	2 (14.3)	2 (13)	8 (50)	17 (35)	12 (25)	16 (32)	43 (40)	30 (28)	37 (34)
4+5	10 (71.3)	9 (60)	8 (50)	26 (53)	27 (55)	29 (58)	49 (46)	54 (51)	59 (55)
<i>Total Observations</i>	14	15	16	49	49	50	107	106	108
C-peptide (nmol/l)									
Fasting C-peptide	0.37±0.19	0.23±0.09	0.26±0.10	0.30±0.17	0.25±0.11	0.26±0.14	0.29±0.18	0.29±0.15	0.28±0.16
Stimulated C-peptide AUC	0.71±0.33	0.61±0.23	0.71±0.21	0.71±0.35	0.66±0.26	0.64±0.24	0.66±0.35	0.68±0.31	0.65±0.30
Glycated hemoglobin (%)	7.07±0.72	7.15±0.91	7.04±1.11	6.84±0.65	6.81±0.91	6.87±1.04	7.10±1.20	6.98±1.05	7.19±1.21
Glycated hemoglobin (mmol/mol)	54	55	53	51	51	52	54	53	55
Insulin dose (IU/Day/kg)	0.64±0.26	0.71±0.27	0.51±0.23	0.59±0.29	0.62±0.31	0.55±0.23	0.54±0.28	0.60±0.35	0.57±0.28
Fasting plasma glucose (mmol/l)	6.81±1.92	6.32±2.15	5.87±1.24	6.28±2.16	5.95±1.74	5.69±1.19	6.31±2.05	6.28±2.05	6.59±2.18
Median GADA (Units/ml)	193	440	219	204	312	190	199	159	237
Median IA-2A (Units/ml)	408	667	943	472	620	736	NA	NA	NA

Values are mean±SD unless stated otherwise. HLA, human leukocyte antigen; 4D, 4 doses of GAD-alum; 2D, 2 doses of GAD-alum; AUC, area under the curve;

GADA, Glutamic acid decarboxylase antibodies; IA-2A, Insulinoma antigen 2 antibodies. HLA risk classification is based on HLA class II type. The Tanner puberty stage ranges from 1 to 5, with higher stages indicating more developed genitalia.

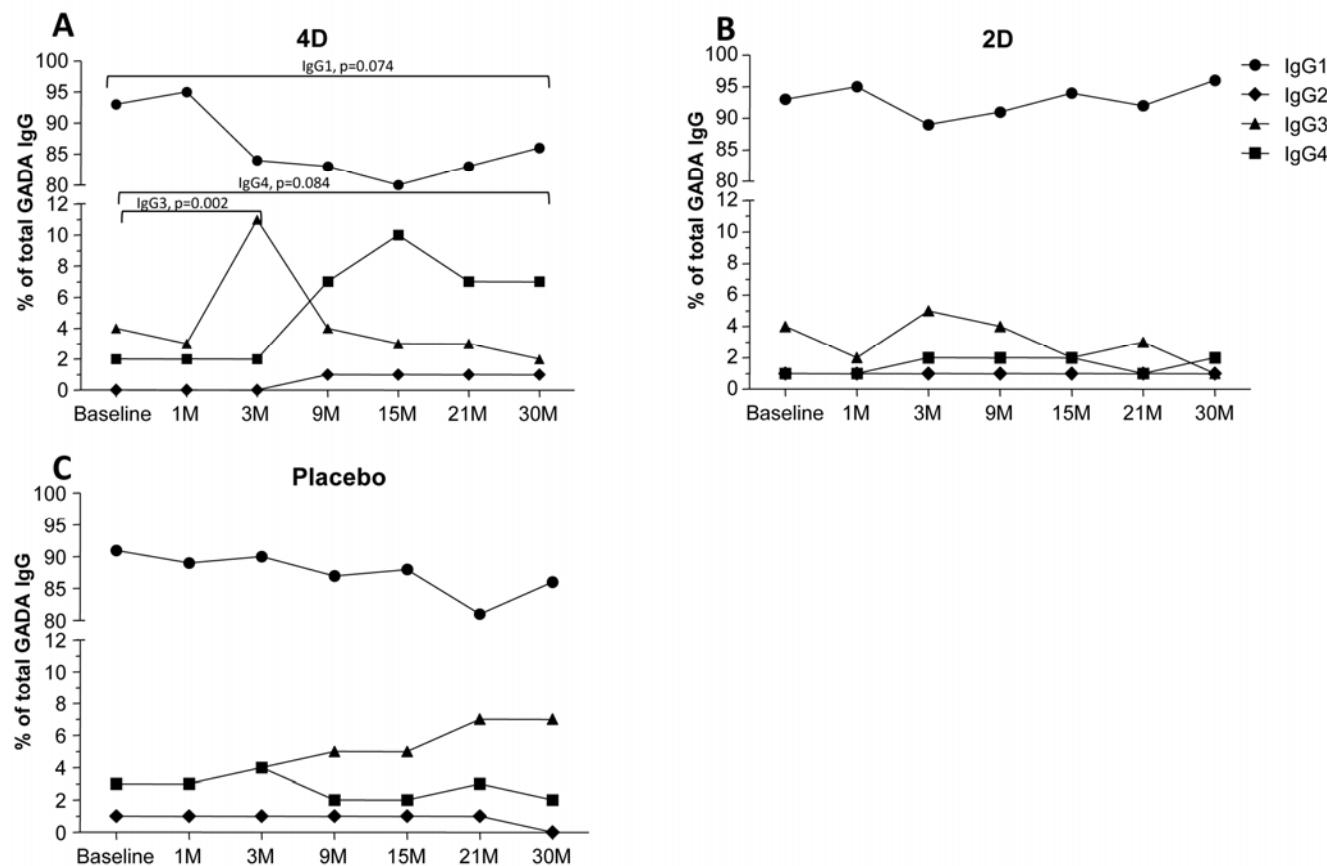
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Supplementary Figure 1. Changes in autoantibody titers. Median GADA titers (A) and median GADA fold-change from baseline (B) in 4D (black circles), 2D (grey circles) and placebo (empty circles). Significant differences are indicated as p-values.



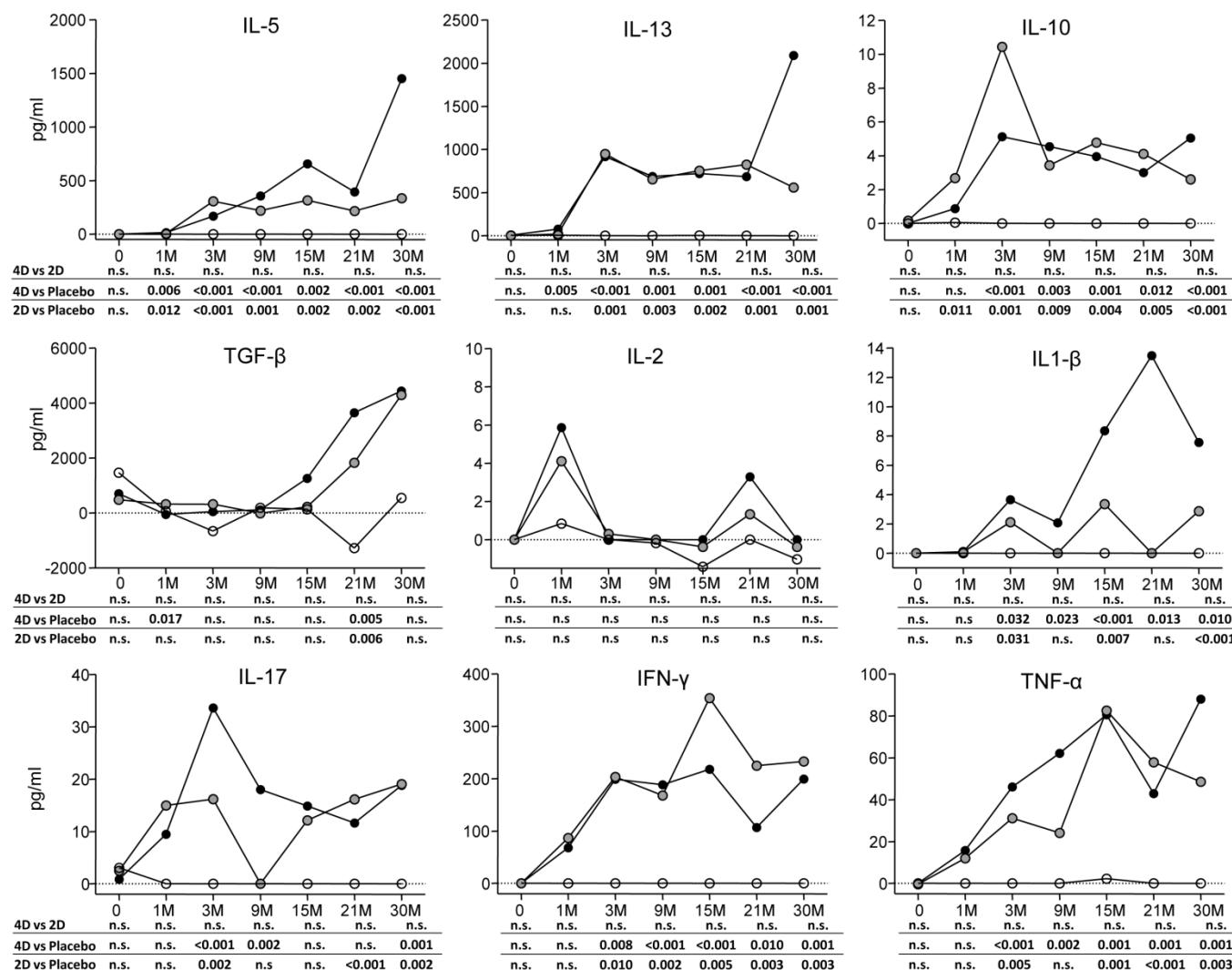
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Supplementary Figure 2. Median GADA IgG1-4 subclass distribution. The GADA IgG subclass distribution presented as a percentage of total IgG for 4D (A), 2D, (B) and placebo (C). Results were expressed as cpm, and positivity of each sample was calculated by subtraction of the mean cpm value plus three times the standard deviation (SD) obtained for the negative control. Significant differences are indicated as p-values.



SUPPLEMENTARY DATA

Supplementary Figure 3. Cytokine secretion upon in vitro PBMC stimulation. Median secretion of IL-1 β , IL-2, IL-5, IL-10, IL-13, IFN- γ , TNF- α and TGF- β (pg/ml) in the subgroup of patients followed for 30 months, detected by Luminex in PBMC supernatants after 7 days culture with GAD₆₅, in patients receiving 4 doses of GAD-alum (4D, black circles), 2 doses of GAD-alum (2D, gray circles) or placebo (white circles). GAD₆₅-induced cytokine secretion is given after subtraction of spontaneous secretion. Significant differences are indicated as p-values.



SUPPLEMENTARY DATA

Supplementary Figure 4. Relative contribution of each cytokine to the GAD₆₅-induced secretion. Bar chart illustrating the relative contribution (%) of each cytokine to the GAD₆₅-induced secretion detected by Luminex from 1 month to 30 months follow-up in patients receiving 4 doses of GAD-alum (4D), 2 doses of GAD-alum (2D) or placebo.

