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Kharagjitsing, A.V.

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Chapter 5

Association of Interferon- γ and Interleukin 10 Genotypes and Serum Levels with Partial Clinical Remission in Type 1 Diabetes

B.Z. Alizadeh[†], P. Hanifi Moghaddam[†], P. Eerligh, A.R. van der Slik, H. Kolb, A.V. Kharagjitsingh[‡], A.M. Pereira Arias, M. Ronkainen, M. Knip, R. Bonfanti, E. Bonifacio, D. Devendra, T. Wilkin, M.J. Giphart, B.P.C. Koeleman, R. Nolsøe, T. Mandrup Poulsen, N.C. Schloot and B.O. Roep, for the DIABMARKER Study Group.

[†]Both authors contributed equally

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Abstract

Introduction: We studied whether serum IFN- γ or IL-10 levels and their corresponding functional polymorphic genotypes are associated with partial remission of Type 1 Diabetes (T1D).

Methods: A multi-centre study was undertaken in patients with newly diagnosed T1D and matched controls. T1D patients were followed for 3 months and characterised for remission status. Partial clinical remission was defined as a daily insulin dose ≤ 0.38 Units/kg/24hr with an HbA1c $\leq 7.5\%$. Thirty-three patients and 32 controls were phenotyped for serum concentrations of IFN- γ and IL-10 and genotyped for functional polymorphisms of the IFN- γ and IL-10 genes.

Results: Sixteen of 25 informative patients (63%) remitted. Serum IFN- γ concentrations were significantly decreased in remitters, but increased in non-remitters compared to controls, and did not change over time in any group. IFN- γ genotypes corresponded with serum levels in controls and non-remitters, but not in remitters who displayed the lowest serum IFN- γ levels despite more often carrying high-producing IFN- γ genotypes. Neither the frequency of IL-10 genotypes nor serum IL-10 concentration differed between patients and controls. The combination of high-producing IFN- γ genotype together with low serum IFN- γ concentration at the time of diagnosis provided a strong positive predictive value for remission.

Conclusion/Interpretation: Serum IFN- γ concentrations predicted by genotype and observed serum levels were discordant in remitters, suggestive of regulation overruling genetic predisposition. Although high-producing genotypes were less frequent in remitters, they were predictive of remission in combination with low serum IFN- γ levels. These data imply that remission is partially immune mediated and involves regulation of IFN- γ transcription.

Introduction

Clinical remission occurs in 50% of patients with Type 1 Diabetes (T1D); it is distinguished by a temporary increase in endogenous insulin secretion, and a reduction of exogenous insulin usage, while maintaining normoglycemia [1,2]. Pathologically, remission may reflect a resolution of islet inflammation [1]. Although several factors such as age, gender, islet antibodies, and immune modulation are associated with clinical remission [1,3-5], the underlying immune regulatory mechanisms are unclear. An approach to identify factors associated with remission is to investigate the determinants of inflammatory responses involved in T1D, which may also influence remission. Th1-like immune responses, which predispose to T1D are characterised by production of interferon gamma (IFN- γ) [6,7]. Conversely, interleukin 10 (IL-10) suppresses Th1-like immune response and plays a regulatory role in T1D [8]. We therefore studied whether IFN- γ or IL-10 serum levels in combination with the corresponding genotypes (IFN- γ and IL-10, respectively) are associated with clinical remission of T1D.

Subjects and methods

Participants

This investigation was nested within the European multi-center Remission (REM) Trial aimed at unravelling the determinants of partial clinical remission in T1D. The medical ethical committees of the participating centers approved the study and informed consent was obtained from the participants. The German and Danish cohorts had DNA samples available to be characterized for candidate genes involved in inflammatory responses, and were thus included in this study. Patients

were diagnosed with T1D according to WHO and the American Diabetes Association criteria and included 3 to 7 days after diagnosis and initiation of insulin therapy. At the time that a patient was newly diagnosed and enrolled in the study, an age- and gender matched unrelated volunteer from the same residential area, was recruited. Participants were Caucasoid without a history of acute allergies, infections, or anti-inflammatory medication. The baseline examination took place at the time of diagnosis in patients, and at the first visit in controls. The participants were re-examined at the end of a follow-up period of 3 months by the same study physician using a standardized questionnaire and checklist. Partial clinical remission was defined as having an HbA_{1c} value $\leq 7.5\%$ and an insulin requirement ≤ 0.38 U/kg/24 hours. The remission status remained undetermined for eight patients of the 33 patients studied, owing to incomplete clinical information (n=3) or loss to follow-up (n=5). Thirty-one controls were included in the study.

Measurements

Standardised blood sampling was performed in fasting subjects. Serum levels of IFN- γ and IL-10 were measured by double-sandwich enzyme linked immunosorbent assay as described [9]. The IFN- γ gene contains a CA-microsatellite repeat in the first intron associated with IFN- γ secretion levels *in vitro* [10]. This marker has five variants defined by the number of CA repeat (i.e. 11 repeats for allele one, 12 for allele two (designated allele 116), 13 for allele three, 14 for allele four and 15 repeat for allele five) [10] and participants were genotyped as described [11]. Genotyping was successful for all controls and 31 patients. IFN- γ genotypes were grouped as non-carriers (presented as X/X), heterozygous (X/116) and homozygous (116/116) for the 116 low-producing

allele [10]. A CA-microsatellite in the promoter of *IL-10* associated with circulating levels of IL-10 [12] was genotyped in participants. This marker has five allelic variants designed as the alleles 194, 196, 198, 200, and 202. *IL-10* genotypes were grouped as homozygous, heterozygous and non-carriers for the *IL-10* 196 high-producing allele. The genotype frequencies of both genes were in Hardy-Weinberg equilibrium.

Data analysis

Patients were analyzed as one group or divided into remitters and non-remitters. Chi-square, Fisher's exact, Student's *t*, ANOVA and Kruskal-Wallis tests were used to compare frequencies and means. Logistic and generalized linear regression analyses were used to estimate and compare the age adjusted mean and the corresponding standard errors of IFN- γ concentrations amongst the groups. In view of the relation between partial remission and genotype and serum IFN- γ levels we assessed the positive predictive values (PPV) of these markers separately or combined for remission as described [13]. PPV refers to proportion of remitters among the patients who possessed the "marker". Here, the word "marker" refers to low levels of serum INF- γ , *IFN- γ* genotypes, or their combinations. All the analyses were performed in the STATA version 8.0 software for Windows (StataCorp LP, College Station, TX, USA).

Results

Sixteen patients out of 25 with complete clinical data (63%) developed partial remission during the follow-up period (Table 1). Remitters were older than non-remitters (mean age $34.3 \pm \text{standard error (SE)} 1.6$ vs. 26.3 ± 2.8 years, $p=0.02$) and controls (30.2 ± 1.2 years, $p=0.04$; Table 1).

Overall, serum IFN- γ levels were not significantly different between controls (15.0 ± 4.4 pg/ml) and all patients (13.1 ± 4.2 pg/ml) at the first visit (Table 1; Figure 1). However, serum IFN- γ concentrations were significantly lower in remitting patients compared to non-remitters or controls (5.7 ± 5.2 vs. 22.9 ± 7.1 ($p=0.04$) and 15.4 ± 4.4 ($p=0.02$) pg/ml, respectively; Figure 1), whereas non-remitters had significantly increased IFN- γ concentrations compared to controls ($p=0.05$). We assessed the functionality of the *IFN- γ* polymorphism, i.e. relationship to serum IFN- γ levels with respect to remission. Among controls, homozygous persons had lower serum concentrations of IFN- γ (5.8 ± 1.4 pg/ml, $n=4$) compared to those heterozygous (12.7 ± 2.4 pg/ml, $p=0.02$, $n=15$) or non-carriers of the *IFN- γ* 116 allele (24.0 ± 11.5 pg/ml, $p=0.04$, $n=12$), confirming the functionality of this polymorphism. The same trend was observed in non-remitters (8.9 ± 2.1 pg/ml, $n=3$; 12.9 ± 6.9 pg/ml, $n=5$; and 98.1 pg/ml, $n=1$, in patients homozygous, heterozygous or non-carrier of the 116 allele, respectively). In contrast, remitters did not showed the same trend as observed in controls and non-remitters. Remarkably, remitters not carrying the 116 allele had lower or equal levels of IFN- γ compared to remitters heterozygous or homozygous for this allele (4.6 ± 1.2 pg/ml, $n=3$; versus 8.6 ± 2.3 pg/ml, $n=7$; and 4.4 ± 0.8 pg/ml, $n=5$; respectively). These observations may imply that regulatory factors in the disease course overrule the *IFN- γ* genotype effects in remitters. Combinations of *IFN- γ* genotype status and serum levels were analyzed as predictive surrogates for partial clinical remission. Eleven out of 16 remitters versus five out of nine non-remitters had a low serum IFN- γ level indicating a PPV of 68.8% for partial remission. PPV for partial remission increased to 83.3% for being carriers of the high-producing *IFN- γ* genotypes When *IFN- γ* genotype

Table 1 | Baseline and genotypic characteristics of the study population

	Controls	Overall	Patients	
			Non-Remitters	Remitters
Numbers	31	33	9	16
German/Danish nationality (n)	15/16	17/16	4/5	8/8
Female gender (%)	32.3	36.4	55.6	37.5
Age (years)	30.2 ± 1.2	31.6 ± 1.3	26.3 ± 2.8	34.3 ± 1.6 ^{††}
Body mass index (kg/m ²)	23.0 ± 0.5	24.0 ± 0.9	21.5 ± 0.6	23.5 ± 0.9
HBA _{1c} (%)	5.3 ± 0.1	11.5 ± 0.3 [†]	12.6 ± 0.8	11.1 ± 0.4
Prevalence of <i>IFN</i> -γ genotypes (%) [*]	n=31	n=31	n=9	n=15
116 homozygotes	12.9	25.8	33.3	20.0
116 heterozygotes	48.4	45.2	55.6	46.7
Non-carriers of 116	38.7	29.0	11.1	33.3
Prevalence of <i>IL10</i> genotypes (%)				
196 homozygotes	38.1	54.8	66.7	46.7
196 heterozygotes	51.6	38.7	22.2	46.7
Non-carriers of 196	9.7	6.4	11.1	6.6

Values represent means±standard error. [†]remitting patients compared to those without remission: p<0.05; ^{††}patients with remission compared to controls: p<0.05; ^{*}patients compared to controls: p<0.05. ^{*}Combination of alleles of the CA repeat marker in intron 1 of the *IFN*-γ gene. The observed genotypes were grouped according to the presence of the 116 allele.

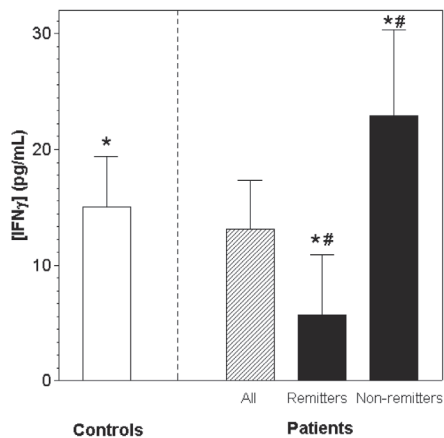


Figure 1 | Levels of serum IFN-γ in remission of T1D. The error bars represent SE. ^{*}p<0.05 remitters or non-remitters compared to controls; [#]p<0.05 remitters compared to non-remitters.

status and serum levels were combined, PPV increased to 100% for co-occurrence of being carriers of the high-producing *IFN-γ* genotypes together with discrepant low circulating concentrations of *IFN-γ* as four out of 16 remitters versus none of the non-remitters had this condition.

In controls (n=31), IL-10 levels were generally low (Table 1). All four patients with increased serum IL-10 levels were homozygous for the *IL-10* 196 allele, but remission status was randomly distributed amongst patients i.e. two patients were non-remitters, one was remitter, and one had an undetermined remission status. In patients with low IL-10 levels, three (27.3%) out of 11 non-carriers, and four (40.0%) out of 10 patients homozygous for the 196 allele remitted (exact p=0.6). Taken together, our observations suggest that *IL-10* genotypes and serum levels may not have a strong impact on the course of disease in this cohort.

Discussion

In the present study, we provide evidence for immunological surrogates for clinical remission. Genetic predisposition to differential levels of serum *IFN-γ* was discordant in remitters, but concordant in non-remitters and controls. This discrepancy marked remission and was not associated with IL-10 abnormalities.

Our patients did not display lower serum levels of *IFN-γ* compared to controls. However, separating patients according to their remission status yielded strong distinction based on circulating *IFN-γ* concentrations, with remitters showing decreased levels compared to controls. This is in line with experiments in NOD mice showing low

levels of *IFN-γ* in parallel with high IL-10 levels, linked to T-cell mediated suppression [14,15]. Remarkably in our study, low serum *IFN-γ* concentrations amongst remitters were consistently accompanied by low IL-10 levels, reflecting a low inflammatory phenotype of the combined cytokines that is consistent with our hypothesis. We propose that genetic predisposition to high *IFN-γ* production in combination with a discrepantly low serum *IFN-γ* concentration independently of IL-10, may predict partial clinical remission in T1D.

Next, we hypothesised that IL-10, an anti-inflammatory cytokine could suppress islet autoimmunity, thereby reducing the need for exogenous insulin and predisposing to clinical partial remission. However, neither serum IL-10 levels nor genotype frequencies differed between remitters, non-remitters, and controls confirming previous reports [16,17]. The patients with high IL-10 production carried the high-producing *IL-10* genotype, but these patients were equally distributed amongst remitters and non-remitters. Taken together, our data suggest that circulating IL-10 or the corresponding genotypes or their combination play a limited role in T1D and hardly contribute to the disease heterogeneity.

Our study has some limitations. A major concern is the small number of subjects limiting the statistical power. An observer related bias is unlikely, as the experiments and genotyping were performed blindly to remission status. To accommodate multiple-testing, we focused on a priori specified immunological candidate markers. These were adjusted for age as a previously identified factor in clinical remission, since we have shown previously that cytokines are useful to predict the individual autoantibody status in patients with newly diagnosed T1D [9]. Finally, the definition

of full clinical remission is open for debate. We chose to study partial clinical remission instead of complete remission, using the definition previously applied to the cyclosporine intervention trial [18].

In conclusion, we demonstrated that *IFN-γ* genotypes in combination with serum *IFN-γ* concentrations are strong predictors of partial clinical remission in patients with newly diagnosed T1D. Our data imply that the immune status in combination with genetic predisposition contributes to partial clinical remission. Our results may prove useful to categorise patients participating in immune intervention trials into subjects as liable to clinical remission or to rapid disease progression.

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References

1. Hramiak IM, Dupre JFinegood DT. Determinants of clinical remission in recent-onset IDDM. *Diabetes Care* 1993; 16: 125-32.
2. Martin S, Pawlowski B, Greulich B, Ziegler AG, Mandrup-Poulsen TMahon J. Natural course of remission in IDDM during 1st yr after diagnosis. *Diabetes Care* 1992; 15: 66-74.
3. Keymeulen B, Vandemeulebroucke E, Ziegler AG, et al. Insulin needs after CD3-antibody therapy in new-onset type 1 diabetes. *N Engl J Med* 2005; 352: 2598-608.
4. Herold KC, Hagopian W, Auger JA, et al. Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. *N Engl J Med* 2002; 346: 1692-8.
5. Hoppu S, Harkonen T, Ronkainen MS, et al. IA-2 antibody isotypes and epitope specificity during the prediabetic process in children with HLA-conferred susceptibility to type I diabetes. *Clin Exp Immunol* 2006; 144: 59-66.
6. Atkinson MAMaClaren NK. The pathogenesis of insulin-dependent diabetes mellitus. *N Engl J Med* 1994; 331: 1428-36.
7. Arif S, Tree TI, Astill TP, Tremble JM, Bishop AJ, Dayan CM, Roep BOPeakman M. Autoreactive T cell responses show proinflammatory polarization in diabetes but a regulatory phenotype in health. *J Clin Invest* 2004; 113: 451-63.
8. Kallmann BA, Lampeter EF, Hanifi-Moghaddam P, Hawa M, Leslie RDKolb H. Cytokine secretion patterns in twins discordant for Type I diabetes. *Diabetologia* 1999; 42: 1080-5.
9. Hanifi-Moghaddam P, Schloot NC, Kappler S, Seissler JKolb H. An association of autoantibody status and serum cytokine levels in type 1 diabetes. *Diabetes* 2003; 52: 1137-42.
10. Pravica V, Asderakis A, Perrey C, Hajeer A, Sinnott PJHutchinson IV. In vitro production of IFN-gamma correlates with CA repeat polymorphism in the human IFN-gamma gene. *Eur J Immunogenet* 1999; 26: 1-3.

11. Eerligh P, Koeleman BP, Dudbridge F, Jan Bruining G, Roep BO, Giphart MJ. Functional genetic polymorphisms in cytokines and metabolic genes as additional genetic markers for susceptibility to develop type 1 diabetes. *Genes Immun* 2004; 5: 36-40.
12. Gibson AW, Edberg JC, Wu J, Westendorp RG, Huizinga TW, Kimberly RP. Novel single nucleotide polymorphisms in the distal IL-10 promoter affect IL-10 production and enhance the risk of systemic lupus erythematosus. *J Immunol* 2001; 166: 3915-22.
13. Khoury MJ, Newill C, Chase GA. Epidemiologic evaluation of screening for risk factors: application to genetic screening. *Am J Public Health* 1985; 75: 1204-8.
14. Schloot NC, Hanifi-Moghaddam P, Goebel C, Shatavi SV, Flohe S, Kolb HR, Rothe H. Serum IFN-gamma and IL-10 levels are associated with disease progression in non-obese diabetic mice. *Diabetes Metab Res Rev* 2002; 18: 64-70.
15. Cardell SL. The natural killer T lymphocyte: a player in the complex regulation of autoimmune diabetes in non-obese diabetic mice. *Clin Exp Immunol* 2006; 143: 194-202.
16. Ide A, Kawasaki E, Abiru N, et al. Genetic association between interleukin-10 gene promoter region polymorphisms and type 1 diabetes age-at-onset. *Hum Immunol* 2002; 63: 690-5.
17. Urcelay E, Santiago JL, de la Calle H, Martinez A, Figueredo A, Fernandez-Arquero M, de la Concha EG. Interleukin-10 polymorphisms in Spanish type 1 diabetes patients. *Genes Immun* 2004; 5: 306-9.
18. Feutren G, Papoz L, Assan R, et al. Cyclosporin increases the rate and length of remissions in insulin-dependent diabetes of recent onset. Results of a multicentre double-blind trial. *Lancet* 1986; 2: 119-24.