Remission Phase in Paediatric Type 1 Diabetes: New Understanding and Emerging Biomarkers

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Keywords

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Abstract

Type 1 diabetes (T1D) is a metabolic disease of unknown aetiology that results from the autoimmune destruction of the β -cells. Clinical onset with classic hyperglycaemic symptoms occurs much more frequently in children and young adults, when less than 30% of β -cells remain. Exogenous insulin administration is the only treatment for patients. However, due to glucose dysregulation, severe complications develop gradually. Recently, an increase in T1D incidence has been reported worldwide, especially in children. Shortly after diagnosis, T1D patients often experience partial remission called “honeymoon phase,” which lasts a few months, with minor requirements of exogenous insulin. In this stage, the remaining β -cells are still able to produce enough insulin to reduce the administration of exogenous insulin. A recovery of immunological tolerance to β -cell autoantigens could explain the regeneration attempt in this remission phase.

This mini-review focuses on the remission phase in childhood T1D. Understanding this period and finding those peripheral biomarkers that are signs of immunoregulation or islet regeneration could contribute to the identification of patients with a better glycaemic prognosis and a lower risk of secondary complications. This remission phase could be a good checkpoint for the administration of future immunotherapies.

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Type 1 Diabetes and Autoimmunity

Type 1 diabetes (T1D) is a metabolic disease caused by the destruction of insulin-producing β -cells by the immune system. It is the commonest endocrine disease in children and adolescents [1], although it can appear at any age. T1D is preceded by a long asymptomatic period (prediabetes) in which immunological tolerance to β -cells is lost. The clinical symptoms of the disease arise after a significant reduction of β -cell mass. Autoantibodies to islet cells can be detected in the sera of subjects at the pre-

The spontaneous partial remission stage in the natural history of T1D

- Unknown aetiology
- No cure, no prevention
- Increasing incidence, mostly in the early childhood

Fig. 1. Natural history of T1D and proposed sequence for remission phase related to β -cell mass.

clinical stage of T1D. In the experimental model of T1D, the non-obese diabetic (NOD) mouse, antibodies and T-cell responses correlated with the autoimmune reaction to the islets and with islet infiltration, insulinitis [2]. Islet cell autoantibodies are the biomarkers of the autoimmune process, and they reflect the attack to islet components. Some of these components are exclusive of β -cells (insulin, ZnT8 [zinc transporter 8], IGRP [islet-specific glucose-6-phosphatase catalytic subunit-related protein]), while others have a broader neuroendocrine expression (GAD [glutamic acid decarboxylase], IA-2 [islet antigen 2], S100 β) or a neuronal expression (GFAP, glial fibrillary acidic protein), an aspect confirmed in experimental models of the disease [3]. Usually, the first to appear are those that react against insulin, and its prevalence is higher in young children.

The cause of T1D is unknown, although genetic and environmental factors are known to increase the risk for its development. Genetic susceptibility factors are mainly determined by the HLA genes, specifically HLA class II haplotypes DRB1*03:01-DQB1*02:01 (DR3-DQ2) and

DRB1*04:01-DQB1*03:02 (DR4-DQ8) that lead to a strong susceptibility [4]. These genes code for proteins found on the cell surface and are related to important immunological functions such as antigen presentation. Environmental factors are also involved in T1D and most of them could act during childhood (e.g., perinatal factors, infections, diet, and stress events).

Clinical symptoms of the disease arise after a significant reduction of β -cell mass. The alterations observed in the pancreas of T1D patients confirm that the immune system attacks β -cells. However, patients retain some ability to regenerate β -cells until decades after the clinical onset [5], although the autoimmune response destroys new β -cells as they regenerate (Fig. 1). Stopping autoimmunity would be key to a successful regenerative therapy. Shortly after the onset of T1D, a temporary and partial spontaneous remission stage, also known as honeymoon phase, can be detected. During this phase, the exogenous insulin requirement decreases to below 50% of the initial levels – and in some cases even insulin independence can be achieved – while near-normal glycaemic control is

maintained. Following this stage, patients will require insulin treatment for life, and in most cases secondary complications will occur. The partial remission phase can be observed in children, adolescents, and adults, with the same symptoms and metabolic features. In this review, we will focus on the childhood T1D remission phase.

T1D and Childhood

Paediatric T1D is especially relevant because the disease interferes with the quality of life of the patients and their families, increasing the risk of secondary complications in the long term. Recent studies in childhood T1D show some epidemiological changes that indicate a rapid increase with age in the incidence of the disease by approximately 3–4% [6, 7]. No incidence differences between boys and girls have been described. In addition, age at diagnosis tends to be advanced specifically in children aged 0–4 years. These changes in a genetically stable population reflect new active environmental factors that increase the risk of autoimmune diseases, including T1D. This is the basis of the hygiene hypothesis that argues that the absence of a range of infections in early childhood (worms, viruses, and other microbes) impairs maturation of the immune system, thereby affecting the lymphocytic repertoire, altering immunoregulation and increasing the risk of T1D [8]. In fact, perinatal environmental exposure is crucial for sensitizing to β -cell autoantigens and for developing a specific gene signature in autoreactive CD4⁺ T lymphocytes [9]. Overall, these data suggest that T1D is the consequence of a multistep process initiated during the early stages of life.

Children with early signs and clinical symptoms of T1D should be diagnosed as soon as possible to avoid diabetic ketoacidosis, the most common cause of death at T1D onset. Younger age and ketoacidosis at diagnosis have been associated with lower stimulated C-peptide levels 1 year after the onset of T1D, reflecting a poorer residual β -cell function in these patients. Additionally, a faster loss of β -cell function has been reported in the youngest children compared to older age groups. Hence, this may represent a rapid and more extensive destruction of β -cells or a reduced capacity to regenerate β -cells in very young children [10].

Childhood T1D exhibits certain differential features when compared to adults: the asymptomatic period tends to be shorter, residual β -cell function is lower, anti-insulin antibodies are more frequent and the association with high-risk HLA types is higher [11]. In addition, the onset

of T1D before the age of 7 is associated with slower intellectual development and cognitive problems, probably due to secondary complications, such as hyper- and hypoglycaemia, which can affect the development of the brain [12, 13].

The Remission Phase in Paediatric T1D

A particularly interesting stage in the natural history of T1D is the partial spontaneous remission phase. This phase begins a few weeks after insulin therapy has been initiated. During this stage the patient's need for exogenous insulin declines by 50%, and near-normal metabolic control is maintained. In a few cases, even temporary insulin independence can be achieved. There is no consensus in the clinical definition of the remission phase, and various interpretations have been proposed. It may be defined as an insulin requirement of <0.5 units/kg of body weight per day and HbA1c <7%, but a new formula combining both values has recently been proposed: "insulin dose-adjusted HbA1c" (IDDA1c) defined as $\text{HbA1c (\%)} + 4 \times (\text{insulin dose in units/kg/24 h})$, which suggests that a value of <9 would indicate partial remission. This definition correlates well with a stimulated C-peptide >300 pmol/l and has been validated in large cohort studies [14–16]. We firmly believe that for several reasons IDDA1c criteria should be the standard for defining the T1D remission phase: (1) it takes into account insulin doses and metabolic control; (2) it displays a good correlation with C-peptide levels; (3) it has been validated in large cohorts of T1D children and adolescents [16]; (4) it has been recommended by the International Society for Pediatric and Adolescent Diabetes (ISPAD) [17]. According to this new definition, the T1D remission phase can be predicted to occur in 61% at 3 months, in 44% at 6 months, and in 18% after 12 months. Previously, the definition of this stage varied according to the insulin dose (ranging between 0.3 and 0.5 IU/kg/day) considered necessary by the different authors for a correct metabolic control and according to the levels of HbA1c (6.5–7.0–7.5% or 48–53–58 mmol/mol) suggestive of a correct metabolic control [14]. However, the accurate definition of the honeymoon phase has generated much uncertainty.

The prevalence of the remission phase in T1D detected in different studies varies widely (30–80%), which partly reflects the use of these different definitions. The honeymoon phase is present in up to 80% of children [18, 19] and suggests some remaining β -cell function after the on-

set of insulin treatment and probably reflects an attempt at islet regeneration in favourable immunomodulatory conditions [20].

Several clinical and metabolic factors have been found to influence the frequency and duration of the remission period, which depends partly on the recovery of β -cell function [21]. The duration of this stage varies from weeks to years with an average of 7 months, and it is usually longer in patients older than 5 years [19]. At present, the predictive role of this phase is unknown. Children with moderate/severe diabetic ketoacidosis at T1D diagnosis are less likely to enter a partial remission phase and their honeymoon phase is shorter [22]. Partial remission in children younger than 2 years is uncommon, thus indicating a more progressive and rapid destruction of β -cells and less residual insulin secretion at the time of diagnosis [18]. An observational study found that males with T1D are more likely to experience partial remission than females (73 vs. 53%) and also for a longer period of time [23]; however, these results have not been replicated. It has also been suggested that the type of insulin treatment may modulate the duration of this phase. A strict glycaemic control either with basal bolus [24] or with an insulin pump [25] has been shown to extend the honeymoon phase.

Residual β -cell function can be assessed by serum C-peptide level measurements. Proinsulin conversion leads to equimolar production of C-peptide and insulin. Therefore, C-peptide can be used to determine endogenous insulin secretion, even after the initiation of insulin therapy [26]. C-peptide concentration can be evaluated in the basal state or stimulated after an intravenous glucagon injection or after a standardized mixed meal [27]. The latter method is the most widely used in clinical research and intervention studies to monitor changes in β -cell function in T1D patients, although an agreement should be reached to standardize this test. Other proposed biomarkers of β -cell function in humans are proinsulin levels in serum or mediators of β -cell stress such as the proinsulin/C-peptide ratio and the measurement of the heat shock protein 90 (Hsp90) [28].

The underlying pathogenic mechanisms of T1D partial remission remain poorly described. It has been suggested that it is likely to be due to a partial β -cell recovery with improved endogenous insulin production and changes in peripheral insulin resistance due to β -cell rest after the onset of insulin therapy [14]. However, recent studies have shown that this explanation seems incomplete [20, 29]. It is currently hypothesized that the honeymoon phase occurs due to a transient recovery of adaptive

Table 1. Main features of the partial remission (honeymoon) phase in children

Spontaneous, temporary, and partial remission of T1D
Insulin dose-adjusted HbA1c (IDDA1c): $\text{HbA1c (\%)} + 4 \times$ (insulin dose in units/kg/24 h), where a value of <9 would indicate partial remission
Average duration of 7 months, longer in patients aged >5 years; exceptionally, temporary insulin independence can be achieved
Prevalence of up to 80%
Metabolic factors: rest of β -cells after insulinization
Immunological factors: enhancement of specific tolerance to β -cells
Model for understanding the pathogenesis of T1D
Optimal checkpoint for intervention
T1D, type 1 diabetes; IDDA1c, insulin dose-adjusted HbA1c.

immune tolerance, but there is a lack of reliable biomarkers that can echo the decrease in the autoimmune aggression, allowing β -cell replication [30]. In this scenario, there would be an increase in endogenous insulin secretion, although the reduction of the islet glucotoxic environment favoured by the onset of insulin therapy may contribute to an improvement in the function of the remaining β -cells. The main features of this interesting stage are summarized in Table 1. However, further research is needed to determine the metabolic and immunological significance of the T1D remission phase especially in children.

Collateral Damage of the T1D Remission Phase and Newly Proposed Biomarkers

Owing to the attempt at islet regeneration and local immunoregulation, new insulin-producing cells may arise during the spontaneous remission phase. However, β -cell regeneration could have a dual role, i.e., increasing endogenous insulin production but promoting the release of autoantigens, thus perpetuating the vicious circle of regeneration and autoimmunity that fits well the proposed relapsing-remitting nature of T1D. These new epitopes may enhance the autoimmune attack, resulting in collateral damage that balances the immune response towards β -cell destruction and a definitive lack of immunological tolerance to self.

The remission phase has generated much interest as a possible target checkpoint for therapeutic interventions in T1D aiming at preserving remaining β -cells but also at identifying subjects with a minor risk of long-term diabe-

tes-related complications. For this reason, there is a need for biomarkers at this stage. Some metabolic biomarkers have been taken into account in some therapeutic interventions [31]. Hence, reliable and specific biomarkers of spontaneous remission are required to identify subjects at risk, to better monitor this stage, and to accomplish future immune interventions.

Known susceptibility factors for developing T1D are mainly genetic; therefore, there might also be susceptibility genes for entering a honeymoon phase. A recent study has suggested that an allele of the prostaglandin receptor EP4 (PTGER4) could be related to the modulation of remission in patients with T1D [32]. Interestingly, this is a risk factor in other autoimmune diseases such as rheumatoid arthritis or multiple sclerosis. The PTGER4 receptor – one of the 4 receptors of prostaglandin E_2 – activates regulatory T lymphocytes [33] and inhibits the autoimmune response in the NOD mouse model [34, 35]. Therefore, this could be induced through an anti-inflammatory and immune-enhancing mechanism that would explain the longer duration of the honeymoon phase.

Apart from genetic markers for subjects at risk, there is an interest in defining immunological biomarkers of this transient remission that involves β -cell tolerance recovery. These biomarkers could be useful not only to confirm the metabolic stage but also as reporters of the immunological status in the remission phase that can be an optimal stage for immune intervention. This has been demonstrated by the efficacy of rituximab (an anti-CD20 monoclonal antibody) in extending the stage up to 2 years [36] and by abatacept (a monoclonal antibody that blocks the costimulatory molecules CD80 and CD86 required for T-cell activation) in reducing β -cell function decline in T1D patients [37]. These studies are controversial, as they are immunotherapies with significant side effects but demonstrate the effect of inhibition of autoimmunity on the recovery of β -cells and endogenous insulin production.

In this regard, peripheral biomarkers that show immune alterations during remission phase are currently being studied. The complete immune phenotype of this stage is not identified but new data suggest interesting changes. Since T1D is a T-cell-mediated disease, different T-cell subsets are expected to modulate the autoimmune reaction thus leading to partial disease remission. A mathematical model predicted that T1D onset is caused by a dominance of T effector lymphocytes over T-regulatory lymphocytes and that the transition to the honeymoon phase over time may be due to an inversion in the frequencies of these subsets [38]. In agreement with these results, the counts of islet antigen-specific IL-10-produc-

ing cells – an important cell subset in T1D – are decreased in peripheral blood in T1D patients during the remission phase. Patients with a greater number of islet-specific IL-10-producing cells at diagnosis have been associated with good glucose control. Hence, it is suggested that these cells may predict good clinical outcomes by a specific regulatory response [39, 40]. Interestingly, a predominant IL-10 cytokine profile (and low IFN- γ secretion) in peripheral blood mononuclear cells was observed in 1 T1D patient with complete remission, with reported β -cell function [41]. Moreover, low serum IFN- γ concentrations at T1D clinical onset provide a strong positive predictive value for partial remission [42]. The role of immunoregulatory factors in the honeymoon phase can also be observed by an alteration in the frequency of T-cell subsets in peripheral blood: CD4⁺CD45R0⁺ memory T cells, activated T-regulatory lymphocytes, and CD4⁺CD25⁺CD127^{high} (indirectly associated with immunoregulation) [43]. These changes are strongly associated with partial remission duration, glucose control, and β -cell tolerance and function. Another trait of T cells related to partial remission is the increased apoptosis of CD4⁺CD25^{high} T lymphocytes from peripheral blood in T1D subjects during the remission phase [44] proposed as a measure of remission related to the suppressive potential of these cells. The potential proposed new biomarkers for this stage of T1D are summarized in Table 2.

These data suggest that the honeymoon stage is the consequence of genetic predisposition and immunological changes that take place in islet microenvironment but are shown in the periphery, mainly in terms of immunoregulatory T-cell subsets and cytokines. However, further research is required to define reliable biomarkers for clinical use. The success of innovative immunotherapies in preventing and even reverting T1D will depend on the implementation of biomarkers of immunoregulation and regeneration that reflect therapeutic efficacy in clinical trials [45]. Some of the aforementioned biomarkers (ratio of CD4⁺ T cells to CD8⁺ T cells, IFN- γ , and IL-6) are already being used in immunointervention in human T1D such as disease prevention studies and islet transplantation and as markers of a risk of developing T1D [46]. However, there are many hurdles in the implementation of immune biomarkers because autoreactive responses are rare in peripheral blood or require ex vivo manipulation. Islet-infiltrating cells or systemic signatures of immunoregulation can be easily obtained in the NOD experimental model of the disease but require confirmation to be translated into a therapy for T1D patients due to significant differences in both innate and adaptive immunity [47].

Table 2. Proposed new biomarkers for the detection of the remission stage

Type	Name	Mechanism	Ref. No.
Genetic	Prostaglandin receptor EP4 polymorphism	Immunomodulation	30
Cell subset	Less islet-specific IL-10-producing T cells	Immunoregulation	48
Cell phenotype	High IL-10 production by islet-specific T cells	Immunoregulation	38
Soluble	High IL-10 serum concentration	Immunoregulation	39, 40
Cell subset	Alterations in T-cell subsets (memory, activated Treg, and CD4 ⁺ CD25 ⁺ CD127 ^{high})	Immunoregulation	41
Cell phenotype	Increased apoptosis of CD4 ⁺ CD25 ^{high} T cells	Immunoregulation	42
Treg, T-regulatory lymphocytes.			

Clinical Perspectives: The Strength of the Remission Phase

An important question remains: could the remission phase be extended for a long period of time or forever? A sustained honeymoon phase (i.e., lasting some years) and even a total remission of T1D have been reported as uncommon phenomena, especially in children [16, 48]. Although immunological changes during remission are poorly characterized [49], an extension of this stage may benefit T1D patients, since residual β -cell function has been associated with a reduction in long-term and acute complications [50]. Moreover, the partial remission phase appears to be an optimal stage for introducing changes in the diet, immunotherapies, regenerative medicine, or innovative strategies aimed at preserving and increasing β -cell mass in T1D patients. It is believed that T1D could revert during the honeymoon phase, following the re-establishment of self-tolerance, thus inhibiting autoimmune destruction. This would allow β -cell mass recovery and an increase in endogenous insulin secretion [51]. Results obtained with the experimental NOD mouse model indicate that changes in the diet at this stage retain β -cell mass [52], suggesting the therapeutic potential at this checkpoint. Therefore, the remission phase is of great immunological and metabolic interest, and its characterization could help to identify patients with better future glycaemic control [39, 49] and fewer secondary complications. Moreover, this stage may also be a good phase for immunomodulation [53, 54].

Few studies have characterized this stage of the disease, and even fewer have evaluated it in children. Therefore, its mechanisms of action remain unknown. However, recent results [39] indicate immunological predictive differences in patients entering this stage that could correlate with the evolution of the disease. Our group carried out the first transcriptome study of human pancreas and islets of diabetic patients [55]. One of these patients was a 16-year-old boy who died 9 months after disease onset. This boy's pancreas showed signs of immunoregulation, increased expression of regenerating genes, and alterations in inflammation and innate immunity gene expression that suggested a remittent-recurrent feedback [30]. Maternal microchimerism, or the presence of cells of maternal origin in the pancreas of T1D patients, may affect partial remission; these cells are enriched in the insulin-positive fraction and may contribute not only to tissue repair but also to autoimmunity or immunoregulation [56]. It would be interesting to determine whether these cells correlate with the duration of the remission phase.

Preliminary data point towards the study of inflammatory, metabolic, immunological, and regenerative molecules to better understand and characterize paediatric T1D spontaneous remission. It would be of great clinical help to identify new biomarkers of this phase that correlate with β -cell regeneration and immunotolerance induction, as it would facilitate immunomonitoring of future therapies introduced during this stage. On the one hand, as in other autoimmune diseases, the identification of possible alterations in leucocyte subpopulations [57,

58] during T1D remission would constitute a reliable and easy biomarker reflecting the cellular immune process of partial remission. On the other hand, the identification of the role of certain molecules related to autoimmunity, inflammation, regeneration, and immunoregulation during the honeymoon phase would help to understand the mechanisms involved in this phase and constitute accurate markers of the underlying process.

This stage of partial remission of the disease can help us to understand the mechanisms by which β -cells are able to partially halt their destruction, although it may already be late for an immunointervention aimed at a T1D reversal. Ideally, immunointervention should be initiated at the presymptomatic stage. Further research is needed to determine the usefulness of the remission phase as a predictive potential in disease evolution and its strength as a feasible stage for the application of future therapies. The honeymoon phase is therefore a stage of partial recovery of β -cell mass and provides an interesting framework to develop approaches to T1D cure.

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