REGULAR ARTICLE

Hyperglycaemia alters thymic epithelial cell function

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Abstract Insulin-dependent diabetes mellitus (IDDM) is considered to be a consequence of unchecked auto-immune processes. Alterations in immune system responses are thought to be the cause of the disease, but the possibility that altered metabolite levels (glucose) can establish the disease by specifically acting on and altering thymus stroma functions has not been investigated. Therefore, the direct effect of hyperglycaemia (HG) on central tolerance mechanisms as a causative agent needs to be investigated.

Introduction Environmental factors play a major role in establishing non-insulin-dependent diabetes mellitus [1,2]. However, the role of environmental factors in the development of type I diabetes (T1D) is more obscure, particularly with regard to health and a non-predisposed background [3].

The non-obese diabetes (NOD) strain of mice is a well-established model system, whereby diabetes development initiates due to reversible insulitis and then proceeds through an irreversible stage, which leads to infiltration and destruction of β cells [4,5]. The process is thought to be initiated by the presentation of autoantigens by dendritic cells (DCs) in local draining lymph nodes of the pancreas [4].

Some evidence has shown that strong psycho-emotional stress and trauma can contribute to disease development, but their effects have mainly been investigated as exacerbating factors in an already established disease [1,2]. In addition, it is well documented that during stress, glycaemic levels rise and persist as long as the stress remains [1,2].

Streptozotocin (STZ)-induced hyperglycaemia (HG) was shown to increase thymocyte survival in ovalbumin (OVA)-specific T cell receptor (TCR)-transgenic mice compared to controls [6]. However, the role of the thymus in this process was not investigated.

Although predisposition to this disease plays a significant role, one recent study has shown that environmental factors can trigger reversible immune alterations in mice with a normal background [7]. Due to STZ-induced pancreatic islet loss, defective immunosuppressive functions of T regulatory cells

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were observed, although they were reversible upon insulin administration [7]. Moreover, alterations in the overall thymocyte composition were observed, where the percentage of CD4+ SP thymocytes was increased at 30 days after HG establishment compared to control mice [7].

The hypothesis

Mechanisms of central tolerance together with mechanisms of peripheral tolerance provide a way for avoiding life-threatening conditions through the control of the appearance or expansion of autoreactive clones in the periphery, respectively [8]. Altered architecture, atrophy and mast-cell infiltration of the thymus in HG conditions have been reported [9]. In addition, implantation of a thymus from NOD mice into normal mice induces disease development [10].

The hypothesis presented here is that HG can be a primary triggering event in insulin-dependent diabetes mellitus (IDDM) development through effects on thymic stroma function and in particular by affecting medullary thymus epithelial cells (mTECs) during negative selection.

Glucose transporters (GLUTs) comprise a group of Na+-independent facilitative glucose transport proteins [11]. Glucose uptake by lymphocytes is known to be a source of energy for cells, but the glucose metabolism pathway also interacts with other pathways, where it can directly and indirectly modulate downstream functions [12]. However, the expression of GLUTs in thymus epithelial cells (TECs), and particularly mTECs, has never been explored, and therefore their role in modulating mTEC functional capacity remains to be determined.

If the proposed hypothesis is true, then it is possible not only that IDDM is a consequence of tolerance breakdown, but also that HG per se can lead to immune dysregulation and disease manifestation. This hypothesis has been suggested in previous work [6]; however, the direct influence of HG on thymic stroma function was not investigated.

In a previous study, antiapoptotic effects of HG on CD4+ CD8+ DP thymocytes as well as the EL-4 thymoma cell line were observed [6]. This investigation was performed in vitro as well as in vivo using surrogate autoantigen OVA transgenic mice. In that study, an increase in the expression of GLUT1–GLUT4 on thymocytes in response to HG was observed [6]. However, the effect of stromal cells in this work was not addressed.

Several questions arise:

1. What types of GLUTs do TECs express? Does HG affect the expression?
2. What is the contribution of different types of GLUTs in mTEC function? If GLUT overexpression affects negative T-cell repertoire selection, which can be assessed using TCR-transgenic mice, then does GLUT overexpression alter tissue-restricted antigens (TRAs) presented in the thymus not only quantitatively, but also qualitatively? Are pancreas-associated antigens present in these conditions and at what extent?
3. What is the contribution of DCs in the process of negative selection under HG conditions? Which of them – TEC or DC (resident or circulating) – is responsible for the outcomes of negative selection under HG conditions?
4. In the case of humans, why does primary insulin administration under HG conditions cause remission and why is the remission reversible? It is widely accepted that after primary insulin administration, a honeymoon phase occurs; the widely accepted basis for it is the rest of beta cells and their subsequent performance for a period of time [13]. However, auto-immune processes are ongoing and the immunologic basis for remission is poorly understood [13]. The contribution of suboptimal glucose levels during insulin therapy to the course of this auto-immune disease is also not fully understood, and the influence of HG on TECs and their function will require further exploration.
5. If altered GLUT expression in thymic stroma causes an auto-immune condition, then will the reactions be directed to the pancreas or occur in a more generalised pattern?

Evaluation of the hypothesis

Beta cells of STZ- or alloxan-treated mice die by irreversible damage due to the generation of reactive oxygen species and the cellular remnants are processed by antigen-presenting cells (APCs), and in particular DCs [13]. However, several experiments have indicated that circulating non-resident DCs can take up autoantigens from the periphery and present them to thymocytes undergoing selection processes in the thymus [14]. Therefore, pancreatic antigens can be provided to thymocytes by circulating DCs in these models as well, and this issue should be considered when these models are used for experimentation. In addition, if stress-induced or alimentary HG models are used, then an excessive amount of circulating insulin can alter the mechanisms of central tolerance [14]. It is also critical to investigate these processes in mouse strains that are not initially predisposed to auto-immune diseases.

The principle of the approach for studying this hypothesis is to imitate GLUT expression levels and the subsequent glucose uptake by TECs to the extent at which it occurs under conditions of HG and investigate the direct cell-autonomous effects of altered glucose uptake on TEC function. One possible experimental approach is based on the assumption that HG causes increased expression of particular GLUTs in thymic stroma cells. This includes:

1) Evaluation of the expression of different GLUTs in thymus stromal cells under conditions of normal glycaemia and HG in connection with pan-cortical (CDR1, Ly51 and β51 [15]) and pan-medullary (UEA-1 and Aire [15]) TEC markers. These differences may be cell-type-specific, and therefore additional investigations of one specific type of TEC (mTEC) will be considered. The most prominent alteration (in particular GLUT expression) will be taken into account and will serve as the basis for further construction of a mouse model.
2) Creation of mice with increased GLUT expression in thymic stroma to imitate specific levels of glucose uptake under HG conditions.

To investigate direct cell-autonomous effects of increased GLUT expression in thymic stroma and to exclude the contribution of secondary processes (i.e., when possible alterations in thymus stroma function are secondary to the effect of HG
on other tissues), it is better to address GLUT overexpression directly on thymic stroma. One approach can be to create transgenic mice with an extra copy of a GLUT gene under the control of the thymus-specific FOXN1 promoter [15]. It is also possible to cross mice expressing Cre recombinase under the control of the FOXN1 promoter with mice expressing the extra copy of a GLUT gene under the control of an inducible promoter with a loxP-flanked STOP cassette, which prevents expression in non-Cre-expressing tissues (i.e., thymus). This will allow for GLUT expression only in the thymus and only at the desirable period of time. Moreover, this will be particularly useful if an age-dependent influence of HG on thymocytes is investigated.

Extra-GLUT may not lead to a high level of GLUT in the plasma membrane, as its trafficking is tightly regulated by insulin and as GLUT expression is regulated at multiple levels [16] or constitutively expressed (e.g., GLUT1) [11]. Theoretically, the level of membrane receptor (if GLUT4 is used; the decision is made on the investigation of GLUT levels on TECs) can be increased if a mutation is introduced into TELEY and FQOI motifs as previously shown [17] (though this was conducted in adipocytes), which can give rise to increased surface expression of GLUT4 on the plasma membrane. To verify successful excision of a target sequence by Cre recombinase, mice bearing ROSA26-YFP can be applied (Fig. 1) [18].

1) Investigation of the functional capacity of thymic stroma. If standard TCR-transgenic (OVA peptide, haemagglutinin-specific, etc.) mice are crossed with strains expressing this antigen not only in the thymus, but also in peripheral tissues, such as under the control of rat insulin promoter (RIP) or ubiquitous promoters, then the results will be generated from the ‘background’ of circulating DCs, which take up such antigens and deliver them to the thymus [6]. Based on a rough approximation, the contribution of circulating DC can be neglected. It is better to use a thymus from neo-self-antigen-expressing mice (experimental mice should be expressing extra-GLUT in the thymic stroma) and transfer it to nude TCR-transgenic mice not expressing the neo-self-antigen elsewhere in order to avoid contribution from the periphery and to observe pure, ‘thymus-resident cell’-mediated negative selection. Alternatively, hybrids from TCR-transgenic mice and mice exclusively expressing neo-self-antigens in the thymus can be used. The readout in either system will be assessed by neo-self-antigen-reactive clones.

Discussion

If the experimental evidence confirms this hypothesis, then the role of HG in priming immune alterations at the level of thymus stroma function will be established.

The degree of irreversibility of alteration in thymic stroma functions depends on the longevity of HG persistence. However, mTECs have a fast turnover [19], and if this is due to altered function, then one question that arises is why reversion of diabetes is not observed after the cells are replaced? It is possible that HG affects TEC precursors with a longer life span or creates an unknown state when TECs of previous generations (affected by HG) influence the functions of the TECs in the next generation, possibly through abnormal directive instructions.

It should be noted that in the proposed approach for evaluating the influence of HG on the TEC, the emphasis is made on OVA-TCR transgenic or other surrogate model systems as readouts. Therefore, the proposed study is intended to explore the phenomenon of these observations, as the molecular mechanisms are obscure. The readout used can include the synthesis of autoantigens as well as their surface expression. Considering the role of autophagy in major histocompatibility complex II (MHCII) loading in TECs [20], it is tempting to speculate the involvement of this process; however, the mechanism by which selectivity of (pro)insulin processing in these circumstances occurs is currently not known. If this process is involved, then the level at which TEC precursors become impaired and the basis for this impairment are also unknown.

We propose that HG should affect an entity that is long-lived in the thymus or must initiate self-propagating pathologic mechanisms. In addition, it seems that the longevity or severity of HG should be sufficient for initiating pathologic processes (e.g., alimentary HG that is limited in severity and duration does not cause diabetes).

This research can be further expanded by analysing more narrowly defined questions, such as how does HG influence auto-immune regulator-mediated gene expression? What is the distribution and density of particular TRAs on mTECs?
Conflicts of interest

We declare that there is no conflict of interest with regard to the content of this article.

Overview Box

What do we already know about the subject?
IDDM is considered to occur as a consequence of unchecked auto-immune responses. The role of HG in the establishment of the disease at the level of direct effects on thymus epithelial cells and their function is not well understood.

What does your proposed theory add to the current knowledge available, and what benefits does it have?
If the proposed hypothesis is true, then it suggests that IDDM is a consequence of not only tolerance breakdown, but also environmental processes, such as stress-induced HG, which can lead to immune dysregulation and disease manifestation. A previous study has provided evidence for increased autoreactive clone survival under HG conditions, which also raised the question of a cause–effect relationship between HG and the auto-immune state [6]; however, the influence of HG on thymic stroma function is unclear.

Among numerous available studies, what special further study is proposed for testing the idea?
The main approach of this study is to imitate specific GLUT expression levels observed under conditions of HG on thymic stroma in order to assess the direct effects on the efficiency of central tolerance mechanisms in various settings.

References