

access to biological analysis for urinary albumin.

However, other screening methods such as the Micral-Test II[®], based on semiquantitative strip assays, have been developed and proven useful in screening for microalbuminuria. Unfortunately, Clinitek[®] Microalbumin has not been evaluated against the earlier Micral-Test II[®] strips.

Furthermore, the decision to use a strip for screening for microalbuminuria is dependent on the cost of the strip and the cost of analysing any positive samples using a biological assay.

Although the Clinitek[®] Microalbumin strip appears to be a useful tool for screening for microalbuminuria, further information is needed before it can be utilized in local diabetes clinics.

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Summary and Comment:

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Thymus, central T cell self-tolerance and type 1 diabetes

Original articles:

Self-antigen-presenting cells expressing diabetes-associated autoantigens exist in both thymus and peripheral lymphoid organs. Pugliese A, Brown D, Garza D, Murchison D, Zeller M, Redondo M, Diez J, Eisenbarth GS, Patel DD, Ricordi C. *J Clin Invest* 2001; 107: 555–64.

Differential splicing of the IA-2 mRNA in pancreas and lymphoid organs as a permissive genetic mechanism for autoimmunity against the IA-2 type 1 diabetes autoantigen. Diez J, Park Y, Zeller M, Brown D, Garza D, Ricordi C, Hutton J, Eisenbarth GS, Pugliese A. *Diabetes* 2001; 50: 895–900.

Summary

In the first paper, the authors characterized individual cells synthesizing major type 1 diabetes-associated autoantigens such as (pro)insulin, glutamic acid decarboxylase and the tyrosine phosphatase-like protein IA-2 in the human thymus and other lymphoid organs

(spleen, lymph nodes). Since these cells also express CD83, CD11c, CD40, CD14, CD80, CD86 and HLA class II, they may be qualified as antigen-presenting cells, mainly dendritic cells (DC) and macrophages. In addition, using the TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling) method, apoptotic lymphocytes were identified in close vicinity to these cells.

In the second paper, the same group describes a differential splicing of the IA-2 mRNA between the pancreas and lymphoid organs (thymus and spleen). Pancreatic islets express full-length mRNA and two alternatively spliced transcripts. Thymus and spleen exclusively express an alternatively spliced transcript lacking exon 13. This exon of IA-2 encodes for transmembrane and juxtamembrane domains that contain several target autoantigenic determinants of type 1 diabetes. This finding identifies a mechanism of gene expression that could play a permissive role in the development of autoimmunity through the absence of tolerance to IA-2 epitopes not expressed in lymphoid organs.

Comment

There is more and more evidence that the intrathymic expression of tissue-specific self-antigens plays a prominent role in the establishment of immunological self-tolerance. In this regard, these two papers lend further support to the concept that the central immunological self-tolerance of β -cell identity may be induced during thymic T cell differentiation [1]. The thymus is a very unique organ and constitutes the only site of permanent confrontation between the repertoire of self-antigens presented by major histocompatibility complex (MHC) proteins and the diversity of T cell receptors for antigen (TCRs) generated by random recombination of gene segments encoding a TCR variable part. The thymus is also the major lymphoid organ located at the crossroads between the immune and neuroendocrine systems. In this organ responsible for thymopoiesis (T lymphocyte generation) [2], the neuroendocrine system regulates the process of T cell differentiation from the very early stages. In addition, inside this organ, which is physically separated from non-self-infectious antigens, T lymphocytes undergo a complex educative process that establishes the central T cell self-tolerance of neuroendocrine principles [3, 4]. Contrary to common assumption, the thymus functions throughout life and plays a fundamental role in the recovery of a repertoire of

competent T cells after intensive chemotherapy or during highly active antiretroviral therapy [5–7].

From the first paper and a number of other observations [1, 8–11], we may draw some definitive conclusions on the intrathymic expression of insulin-related genes (*INS*, *IGF1* and *IGF2*). The members of this family are all expressed in the thymus environment according to a precise hierarchy and topography: *IGF2* (thymic epithelial/nurse cells [TEC/TNC]) > *IGF1* (thymic macrophages) >> *INS* (thymic DC). At the protein level, this hierarchical pattern is of great importance since the tolerogenic response primarily concerns the dominant epitopes of a protein family [12]. Contrary to (pro)insulin, the blockade of thymic insulin-like growth factor (IGF)-mediated signalling, at the level of the IGF ligands (in particular IGF-II) or IGF receptors, severely interferes with the early stages of T cell differentiation [13].

In the second paper, a regulation in the establishment of immunological self-tolerance is suggested by the very interesting observation of a differential splicing of IA-2 mRNA between thymus

and pancreas. Such a difference explains why some important antigenic determinants derived from IA-2 proteins are not tolerated and become strongly autoantigenic. Such shaping of the self-reactive T cell repertoire has already been shown for a splice variant of a proteolipid self-protein expressed in the murine thymic epithelium [14, 15]. The preprotachykinin A gene (*PPT-A*) is also transcribed by rat TEC, but thymus-specific mRNA processing gives rise to only one of the *PPT-A*-encoded peptides (neurokinin A) and not the other (substance P) [16]. With regard to the neurohypophysial genes, oxytocin (*OT*) and vasopressin (*VP*), both *OT* and *VP* transcripts are detected in TEC/TNC from different species. At the peptide level, however, thymic *OT* concentrations are much higher than *VP* concentrations. Consequently, more and more observations suggest the existence of thymus-specific post-transcriptional mechanisms [17].

Clinical and pharmacological implications

As already hypothesized by Burnet in 1973 [18], the pathogenesis of autoimmune diseases could form the appearance of ‘forbidden’ self-reactive clones in the peripheral lymphocyte repertoire.

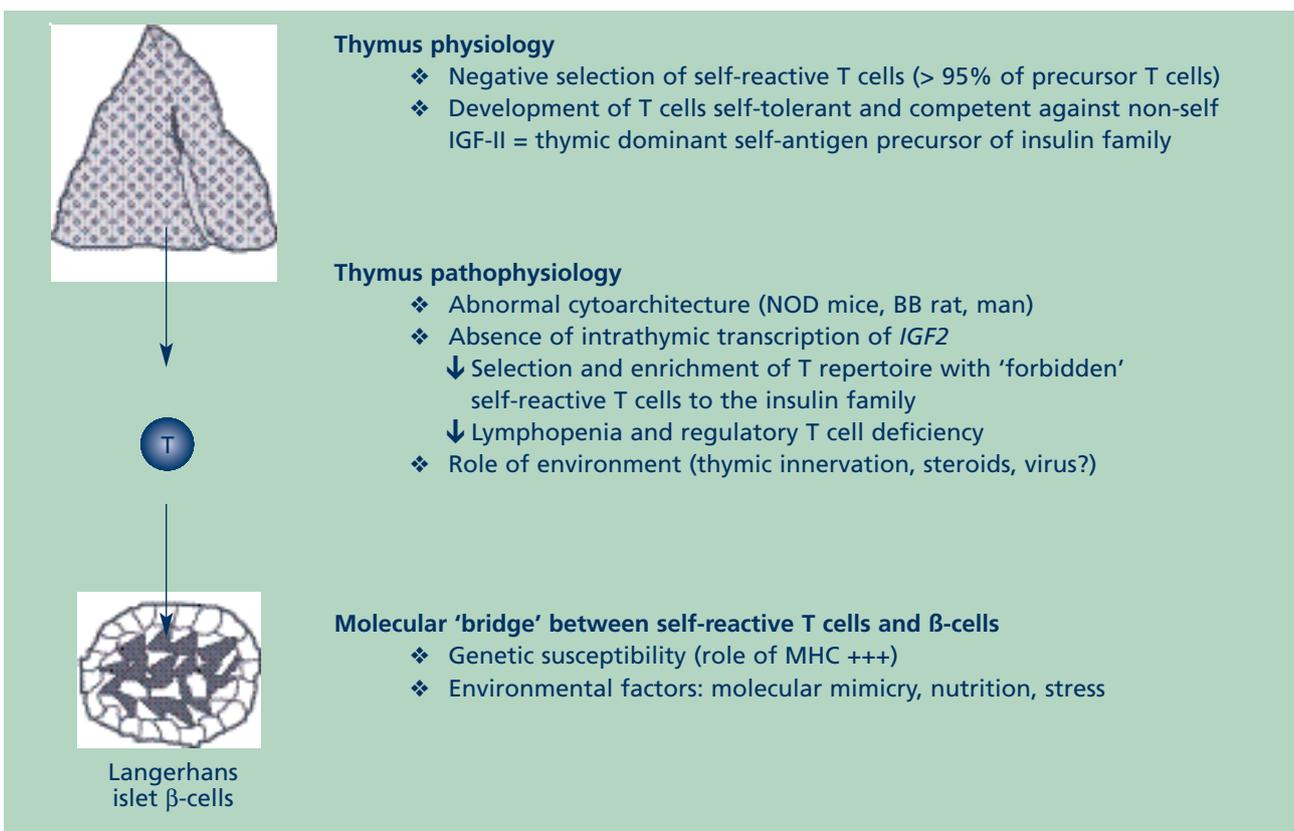


Fig. 1: Role of the thymus in the development of the autoimmune response against islet insulin-secreting β-cells. Together with abnormalities of thymic cytoarchitecture, the thymus-specific defect of *IGF2* expression is responsible both for lymphopenia (with decreased regulatory T cells) and for the absence of central T cell self-tolerance of the insulin family. The influence of environmental factors (such as enterovirus infection) could be exerted at the level of thymic T cell differentiation, as well as in the periphery through the establishment of a bridge between the β-cell autoantigen and ‘forbidden’ self-reactive T cells. NOD, non-obese diabetic; BB, bio-breeding. (Reprinted from [23] with permission from Bentham Science Publishers.)

Since the thymus is the primary site for induction of self-tolerance, thorough investigation of a defective thymic censorship should provide the scientific community with important keys to understand the mechanisms underlying the development of autoimmune responses. A number of abnormalities in thymic morphology and cytoarchitecture have been described for several autoimmune disorders. In accordance with this hypothesis, *INS* transcripts were measured at lower levels in the thymus of human fetuses with short class-I VNTR (variable number of tandem repeats) alleles, a genetic trait of type 1 diabetes susceptibility [19, 20]. The expression of insulin-related genes was also analysed in the thymus, liver and brain of a common animal model of type 1 diabetes, the biobreeding (BB) rat. A thymus-specific defect of *Igf2* expression was evidenced in more than 80% of diabetes-prone BB rats (BBDP) [21]. This defect explains both the lymphopenia (including a lack of antigen-specific regulatory/suppressive T cells generated in the thymus [22]) and the absence of central self-tolerance of insulin family in BBDP rats. As a consequence of the defective thymic censorship, self-reactive T cells bearing TCRs oriented against dominant epitopes of insulin-related peptides continuously migrate from the thymus to the periphery with a poten-

tial cytotoxic power against the islet β -cells. Under certain environmental influences, a molecular ‘bridge’ could be installed between the target autoantigenic epitopes leading to activation of such a self-reactive T cell pool and subsequent β -cell destruction (*Fig. 1*).

The investigation of neuroendocrine genes expressed in the thymus has led to the identification of *neuroendocrine self-peptides*. Through the study of insulin-related gene expression in the thymus, IGF-II — a prominent fetal growth factor — was identified as the dominant self-peptide precursor of the insulin family expressed in the thymus. This observation is in close accordance with the theory of *self-recognition* which, according to F.M. Burnet, is not an inherited property but is gradually acquired in the course of fetal life. Although the tolerogenic properties of neuroendocrine self-peptides remain to be further documented, they are strongly suspected from what is known about the tolerance of classic hormones. The development of specific antibodies by active immunization (or experimentally induced breakdown of tolerance) revealed that tolerance of IGF-II is higher than of IGF-I, and much greater than tolerance of insulin. Insulin is a primary autoantigen tackled by the autoimmune response observed in type 1 diabetes and this could result from its very low

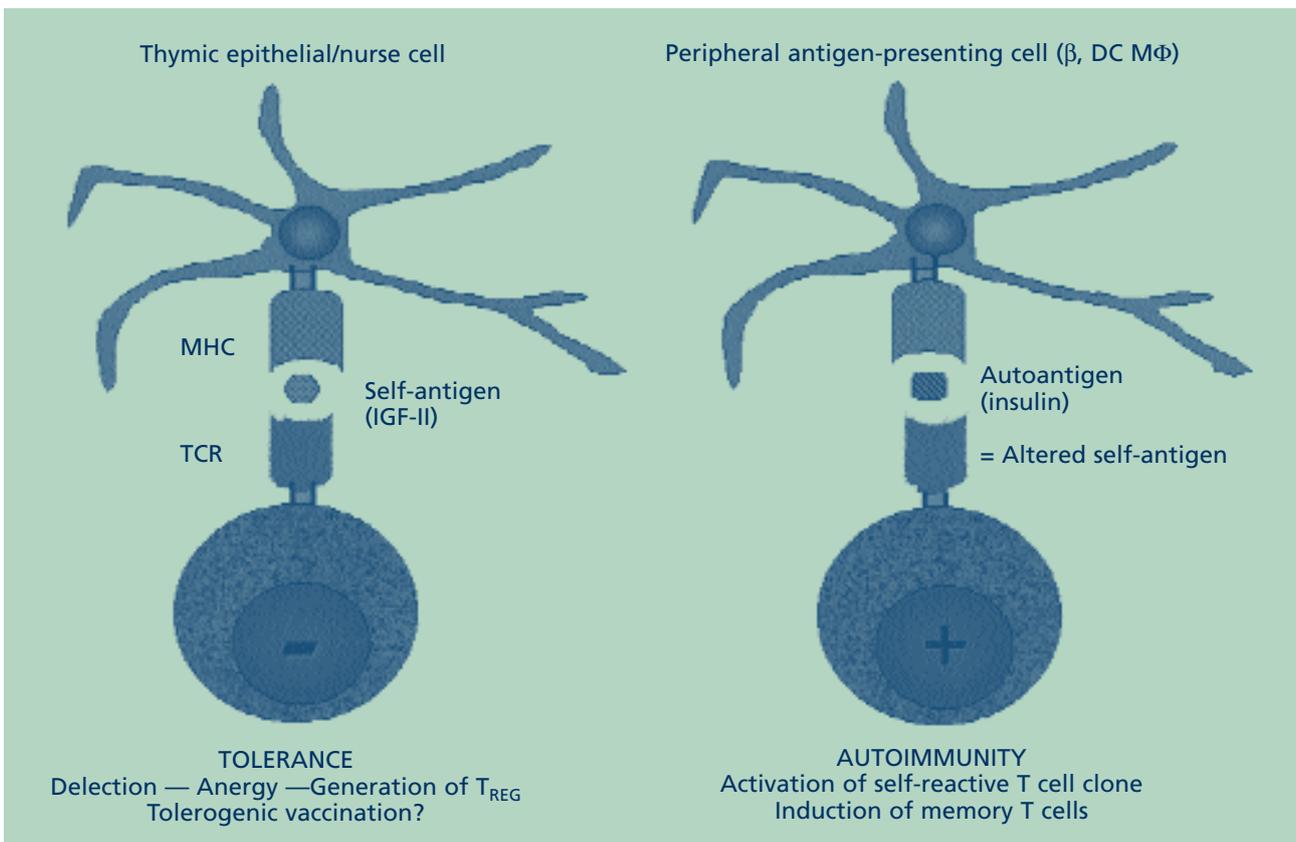


Fig. 2: Pharmacological perspectives: two opposing immune responses driven by two types of antigens. T_{REG}, regulatory T cell.

expression in the thymus. Moreover, autoimmunity has never been observed against IGF-II, and the strong tolerance of this protein, linked to its high expression in the thymus, can be considered as a consequence of the evolutive pressure to protect a fundamental process such as fetal development.

Thus, while insulin behaves as an immunogenic autoantigen of the insulin gene family, IGF-II is the tolerogenic self-peptide precursor of this family. It is now probably appropriate to establish a distinction between an *autoantigen* and a *self-antigen*. According to this distinction, an autoantigen can be considered as an 'altered' self-antigen. Though they are highly homologous, they are not absolutely identical and this biochemical difference theoretically drives opposing immune responses (i.e. immunogenic vs. tolerogenic responses) (Fig. 2). The powerful tolerogenic properties of the thymus should soon be exploited to cure and/or prevent severe autoimmune diseases, such as type 1 diabetes, which constitute the heavy toll paid for the diversity and efficiency of the immune system.

Acknowledgments

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Summary and Comment:
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