Peripheral-antigen-expressing cells in thymic medulla: factors in self-tolerance and autoimmunity

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The thymus expresses many genes previously thought to be specific for cell types in other organs. Thus, insulin genes are expressed in rare cells of the thymic medulla. Thymus transplantation demonstrates a functional capability of such expression for self-tolerance induction. Correlative studies suggest that impaired thymic expression confers susceptibility to autoimmune disease.

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Current Opinion in Immunology 1998, 10:656–662

http://biomednet.com/elecref/0952791501000656

 ISSN 0952-7915

Abbreviations

APC	antigen-presenting cell
CTL	cytotoxic T lymphocyte
EAU	experimental autoimmune uveoretinitis
GP	glycoprotein
IDDM	insulin-dependent diabetes mellitus
IRBP	interphotoreceptor retinoid-binding protein
LCMV	lymphocytic choriomeningitis virus
NOD	nonobese diabetic
NP	nucleoprotein
PAE	peripheral-antigen expressing
PCR	polymerase chain reaction
RT	reverse transcriptase

Introduction

The observational principle of self/non-self recognition by the immune system, in the form of the capability to mount immune responses to foreign antigens while maintaining tolerance/nonresponsiveness toward self antigens, is now founded in compelling experimental fact. The cellular mechanisms of this discrimination are centered on T lymphocytes; these develop and differentiate in the thymus, where they acquire the capability to recognize antigen and acquire functional diversity through the rearrangement of TCRs and the selection of those receptors for the recognition of peptide–MHC complexes. Beyond guiding the development of T cells that manifest recognition of MHC and functional diversity ($CD4^+$ and $CD8^+$ T cells and distinct subsets thereof), the thymus has long been recognized as an effector of self tolerance. Over the past decade a series of elegant experiments has unambiguously demonstrated that the thymus serves to confer nonresponsiveness toward abundantly expressed self antigens. Thus, developing T cells are screened firstly to ascertain that they show sufficient binding to an MHC–peptide complex to be able to provide specific recognition (positive selection) and secondly to cull out those whose binding affinity exceeds an activation threshold that could trigger self reactivity (negative selection). In the latter process, cells are programmed either to die by apoptosis (clonal deletion) or to become functionally paralyzed, incapable of activation (clonal anergy). It is only after maturation and emigration from the thymus to the peripheral lymphatics that T cells can become sustainably activated in response to recognition of peptides through interaction with MHC–peptide complexes on antigen-presenting cells (APCs).

The thymus is organized into two distinctive cellular compartments, the outer cortex and the inner medulla. Each is composed of epithelial cells originating from the brachial arches; the medulla is also rich in hematopoeitic cells which emigrate and associate with the epithelium (primarily medullary); dendritic cells and macrophages are the most readily recognized of the non-T cells among the emigrants. Considerable experimental evidence indicates that the cortex guides positive selection, while the medulla serves to 'negatively select' and thus eliminate — by programmed cell death or by anergy — those T cells that not only recognize MHC–self-peptide complexes but also become activated by that interaction.

There is increasing clarity to the so-called 'central tolerance' mechanism that establishes nonresponsiveness toward the self-MHC molecules and abundantly expressed self peptides they present [1,2]. In contradistinction, mechanisms of induction of 'peripheral tolerance' toward self antigens — that are not so abundant and often are restricted to one or a few organs in the body — remain rather opaque [3,4•,5]. The existence of peripheral tolerance has been inferred from the infrequent but devastating occurrence of organ-specific autoimmune diseases, of which type 1 diabetes and thyroiditis are two clear examples. Since the target cell types attacked by the immune system in these diseases are rare and since autoantigens that are selectively expressed in these rare cell types have been identified and implicated in disease pathogenesis, the notion has developed that distinctive peripheral tolerance mechanisms exist for such rare cell types. Experimental evidence increasingly reveals a complexity to this peripheral tolerance of self; four strategies are currently evident (Table 1). First, tolerizing processes can act in the peripheral lymphoid organs where professional APCs reside for the purpose of activating T cells to marshal immune responses against foreign antigens. The choice between activation or tolerization of a T cell, upon presentation (by the MHC) of a TCR-stimulating peptide, may depend on the maturation stage of the emigrated T cell. Second, T cells may interact with 'amateur' APCs in tissues; this leads to anergy or death of the T cells due to the lack of appropriate co-stimulatory molecules on these APCs (and/or lack of proximal co-stimulatory leucocytes). Third, T cell indifference to (or ignorance of) antigen is thought to effect a form of peripheral self tolerance; here, T cells are neither

Table 1

Peripheral tolerance mechanisms toward rare self antigens.

Mode of tolerance	Cells that induce tolerance	Rationale
Deletion or anergy	Professional APCs in spleen or lymph nodes	Emigrant T cells are initially immature
Anergy	Amateur APCs in tissues	Lack of co-stimulation
Indifference	APCs (professional or amateur)	Weakly activating peptide or low levels of self antigens
Thymic deletion/ anergy (or sensitization?)	Thymic PAE cells	Removal of highest affinity self-reactive T cells?

tolerized nor activated upon encounter with self antigens. In this case the presence of T cells recognizing a tissue antigen can be demonstrated by experimental immunization that induces an immune response (indicating nontolerance), despite the fact that no spontaneous autoreactivity is evident normally. Here the lack of tolerance or of autoreactivity has been attributed either to low levels of the antigen or to weak stimulatory effects upon peptide presentation, by the MHC, to TCR (too weak to induce either anergy or activation). Fourth, while the focus of the field has largely been on clarifying and delineating the three previous strategies and their roles in effecting tolerance toward antigens expressed in specific organs [3,5], a series of clues has led to the realization that the thymus is also contributing significantly to such tolerance of self; this represents another strategy. Here, I review the data which reveal that cells in the thymus ectopically express genes previously thought to be cell-type-specific for other organs, in particular those organs that are susceptible to autoimmune disease; I also consider the ramifications of this thymic expression of peripheral antigen genes for self tolerance and autoimmunity.

Thymic expression of organ-specific genes and identification of PAE cells

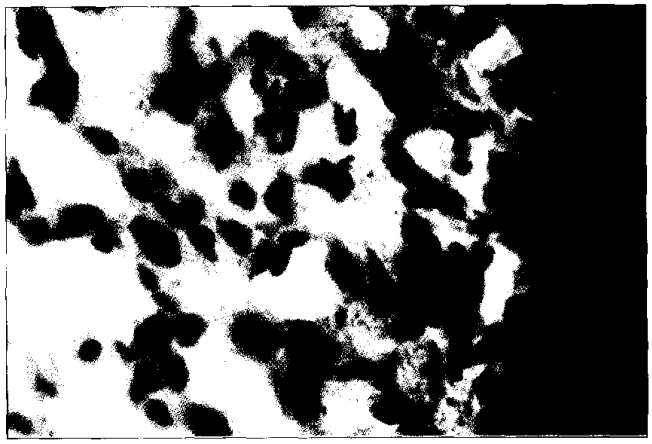
One line of clues about thymic expression of organ-specific genes came from transgenic mice, in particular ones that utilized an insulin-gene regulatory region (i.e. the insulin-gene promoter, RIP) to target gene expression to the pancreatic β cell. Among the motivations for studying the β cell was its involvement in autoimmune type I diabetes, whereby the β cells are selectively destroyed by the immune system. Three groups observed insulin-promoter-driven transgene expression in the thymus [6–8]. Others have reported expression in the thymus of transgenes utilizing gene regulatory elements for mammary-gland lactalbumin [9], erythroid cell β globin [10] and exocrine pancreatic elastase [11]. These observations were initially interpreted with caution since there had been reports describing unexpected patterns of transgene expression that was attributed to the fusion of heterologous gene promoters and coding regions, to the random chromosomal positions of transgene integration [12] or to a low-level nonspecific tissue promiscuity of gene regulation [13]; however, when it was questioned whether thymic expression of an insulin transgene was representative of the endogenous insulin genes, the answer was affirmative. Both mouse insulin genes were found to be specifically expressed in the thymus but not other tissues of ordinary (nontransgenic) mice, using a rigorous polymerase chain reaction (PCR) method to avoid cross-contamination [8]; notably, highly sensitive detection was also required —indicative of low levels of expression. Further, two groups have documented expression of insulin genes in the human thymus [14••,15••] while a third has shown expression of insulin in the rat thymus [16•] and a fourth has confirmed expression in the mouse thymus [17•]; moreover, expression of proinsulin protein by radioimmunoassay was documented using either human or mouse thymi [14••,17•], indicating that the transcription of the gene was indeed producing mRNA that was translated into protein.

The insulin gene had been thought of as the prototypical cell-type-specific gene, expressed only in a rare pancreatic neuroendocrine cell type and in a few brain neurons. The realization that insulin was expressed in the thymus led to an examination of other 'pancreas specific' genes [8]. Both glucagon and pancreatic polypeptide, which define the islet α and PP cell types respectively, were also expressed in thymus as mRNA; each was thought to be specific for a distinct pancreatic cell type and a few brain neurons. Somatostatin, a secreted modulatory peptide which identifies the islet δ cell type, is quite broadly expressed in tissues including the thymus; furthermore, there have been reports of variable expression in human thymus of several other autoantigens implicated in insulin-dependent diabetes mellitus (IDDM), including GAD (glutamic acid decarboxylase)65/67. In addition, a number of exocrine pancreatic gene transcripts — including trypsin, chymotrypsin, amylase, carboxypeptidase A1 and elastase — have been detected in mouse thymus by reverse transcriptase (RT)-PCR [8,11]. As genes that characterize other organs are now being examined, many have been found to be transcribed in the thymus; these include myelin basic protein [18,19], the retinal-specific genes encoding arrestin and interphotoreceptor retinoid-binding protein (IRBP) [20••], the astrocyte gene encoding S100β [21•], the thyroid-specific gene encoding thyroglobulin [16•], the trophoblast gene encoding HLA-G [22•] and liver acute-phase response genes encoding C-reactive protein (CRP) and serum amyloid protein (SAP) [23••].

Characteristics of thymic cells that express organ-specific genes

Immunostaining analyses of both mouse [24••] and human thymi [25] have revealed cells in thymic medulla expressing insulin and, in the case of RIP–Tag transgenic mice, expressing a hybrid insulin transgene producing a novel neoantigen — SV40 T antigen (Tag). The insulin-express-

Figure 1



Proinsulin+ PAE cells in human thymus (magnification approximately 750 ×). Immunostaining (scarlet) for proinsulin visualizes one type of PAE cell, examples of which can be seen here in the thymic medulla and in the cortico-medullary junctional region (to the right of the figure). Often the PAE cells can be found centred within lymphocyte rosettes. Frozen sections (from a six-month-old boy who died accidentally) were fixed in Bouin's solution, reacted with a proinsulin-specific monoclonal antibody, visualized with a secondary antibody coupled to peroxidase – using 3-amino-9-ethylcarbazole (AEC) as a chromophore – and counter-stained with haematoxylin. This photograph is presented courtesy of Alberto Pugliese, Diabetes Research Institute, University of Miami Brain and Tissue Bank for Developmental Disorders (National Institute of Child Health and Development contract NO1-HD-3-3199).

ing cells are relatively large and quite rare. Serial sectioning of entire mouse thymi indicated that only a few hundred cells expressed insulin in each thymus. There are perhaps several thousand insulin-expressing cells in the human thymic medulla, which are often found in the center of lymphocyte rosettes (A Pugliese, personal communication) and thus suggestive of an antigen-presenting interaction. Figure 1 illustrates insulin-expressing cells in human thymus. While these cells are larger than lymphocytes, their identity and cellular lineage remain unclear and the data are somewhat contradictory. The most likely candidates are either bone-marrow-derived nonlymphoid cells (e.g. dendritic cells) or medullary epithelial cells. Using density-gradient cell sedimentation, Smith *et al.* [24••] found that thymic insulin-expressing cells could be segregated into a 'low-density fraction' that is enriched for dendritic cells. Depletion using antibody-coupled magnetic beads was used to demonstrate that peripheral-antigen expressing (PAE) cells expressed class II MHC, consistent with antigen-presenting function. Several other 'islet-specific' genes had a broader fractionation profile — including the low-density fraction as well as others — indicative of more abundant cells or of multiple cell types. Throsby *et al.* [17•] reported that insulin-expressing cells in the low-density fraction were depleted with an antibody (N418) that is selective for dendritic cells, while cells expressing glucagon, somatostatin and pancreatic polypeptide were depleted by an antibody (F4/80) that is specific for macrophages.

The thymic-cell-fractionation experiments are consistent with the islet-antigen-expressing cells being of bone marrow origin, perhaps encompassing several cell types; however, our laboratory has failed to detect Tag-expressing cells in the thymus when RIP–Tag2$^+$ bone marrow was transferred into lethally irradiated nontransgenic recipients (D Daniel, D Hanahan, unpublished data); moreover, work with other peripheral genes argues for an epithelial origin of PAE cells. Thus, Sponaas *et al.* [26], using bone-marrow-chimeric mice, inferred that thymic bone-marrow-derived cells expressed β globin (not surprisingly) but not lactalbumin and that both genes were expressed in radio-resistant (thymic epithelial) cells; furthermore, Klein *et al.* [23••] have shown that two liver acute-phase-protein genes are expressed in a thymic cell fraction that is enriched for medullary epithelial cells but not in a fraction that is enriched for bone-marrow-derived cells. Thus, at this time both lineage relationships and cell type(s) of PAE cells remain ambiguous. What is clear is that reports of cells in the thymus expressing a number of genes that were thought to be organ-specific are increasing. In the case of insulin, the prototypical representative product of a rare cell type and itself an autoantigen, two groups have independently localized the cells expressing it to rare cells in the thymic medulla — consistent with a role in tolerizing developing T cells. We have dubbed the thymic insulin-expressing cells as one of a class of PAE cells, for two reasons: first, their emerging characteristics argue that they represent a discrete sub-cell-type or discrete sub-cell-types; and second, the PAE cells have a demonstrable tolerizing capability, as discussed below, which argues that these thymic cells contribute to the establishment of tolerance toward organ-specific gene products which we have heretofore considered to be manifested in the periphery.

Thymic transplantation indicates functional contributions of PAE cells to self tolerance

The expression of the insulin genes, of insulin-promoter transgenes and of other pancreatic and 'organ-specific' genes at low levels in rare cells of the thymus raises the obvious question of functional significance. Is this a leaky transcription without purpose or a deliberate expression that affects the immune system? Transgenic mice expressing hybrid transgenes controlled by RIP have been employed to address this question, exploiting the fact that such mice can be fully syngeneic with nontransgenic mice except for a single, defined gene product (Table 2). In one example, Miller and co-workers [6] transplanted thymi from RIP–H2-K^b, bm1 mice into nontransgenic bm1 mice (bm1 mice carry a mutant allele of H2-K but are otherwise H-2^b). The recipients of thymus transplants, which could only express H2-K^b therein, accepted subsequent skin grafts from H-2K^b mice; such grafts would otherwise have been rejected. Interestingly, this graft tolerance waned over time. A second experiment utilized RIP-transgenic mice expressing the lymphocytic choriomeningitis virus (LCMV) glycoprotein (GP) or nucleoprotein (NP) antigens [7]. When thymi that

Table 2

Thymic transplants demonstrate tolerizing capability of insulin-PAE cells.

Thymic donor strain	Athymic recipient	Phenotype of recipient mice
RIP–H-2K^b	Thymectomized bm1(H-2K^{mut} otherwise H-2^b)	Tolerant of H-2^b skin grafts; this wanes over time
RIP–LCMV-GP	H-2^b nu/nu	Impaired CTL response after LCMV challenge
RIP1–Tag2; RIP3–Tag2	H-2^b nu/nu	Nonresponsive in humoral compartment to Tag protein; variably tolerant in CTL compartment to Tag$^+$ cells

Table 3

Correlation of thymic expression of insulin- or insulin-promoter-transgenes with resistance to autoimmunity.

In humans

IDDM2 allele	Insulin mRNA in thymus	Insulin mRNA in pancreas	Effect on autoimmunity
Class I	+	+++++	Susceptible
Class III	++	++++	Resistant

In insulin-promoter-transgenic mice

Transgenic line	Transgene mRNA in thymus	Transgene mRNA in pancreas	Effect on autoimmunity
RIP–LCMV-NP	–	+++	Highly susceptible (after LCMV infection)
RIP–LCMV-GP	+	+++	Partially resistant (after LCMV infection)
RIP1–Tag5	–	– → +++	Susceptible
RIP1–Tag2	+	+++	Resistant

expressed NP/GP were transplanted into athymic mice, tolerance toward subsequent challenge with LCMV was induced. In contrast, when thymi from mice that expressed pancreatic but not thymic GP/NP were used, the recipients of thymus transplants were fully responsive. Interestingly, the former mice were only partially tolerant to the transgene product expressed exclusively in their thymi; killing by cytotoxic T lymphocytes (CTLs) was reduced to about 30% of controls. A third example utilized RIP–Tag mice that express Tag in islet β cells and thymus. Previous analyses had demonstrated that RIP–Tag2-transgenic mice were systemically tolerant of Tag in both humoral and cytotoxic compartments [8]. When RIP–Tag thymi from two independent lines of mice were transplanted into athymic mice, the resultant recipient mice were partially tolerant of Tag. The RIP–Tag thymic recipient mice were systemically tolerant of Tag in the humoral compartment but only variably tolerant of Tag in the cytotoxic compartment [24••].

Transplantation of transgenic thymi into athymic mice has also been used to demonstrate a similar tolerizing capability for transgenes controlled by gene regulatory regions of lactalbumin and β-globin [26], elastase [11] or C-reactive protein [23••] thereby supporting the generality of this central component to 'peripheral' organ-specific tolerance mechanisms. Collectively these experiments make a compelling point — thymic expression of neoantigens under the control of the tissue-specific gene prompters can affect immunological development, eliciting partial self-tolerance. In the case of insulin, it is evident that expression in a few hundred thymic PAE cells contributes to tolerance induction. This thymic expression, at least for three insulin-promoter-transgenic neoantigens, cannot evoke complete systemic tolerance in both humoral and cytotoxic compartments. In all three examples, the CTL tolerance resulting from exclusively thymic expression was incomplete. It seems likely that the tolerization initiated by thymic PAE cells toward organ-specific antigens is normally complemented by processes occurring in the periphery, either in the organs themselves or in their draining lymph nodes. Consistent with this notion, Förster and Lieberam [27] have presented evidence for tolerizing events occurring in the pancreatic lymph nodes of RIP–Tag transgenic mice.

Genetic evidence that PAE cells serve to attenuate autoimmune susceptibility

Human genetics has presented a provocative case for the significance of thymic expression of the insulin gene. The two groups that documented thymic expression of insulin in humans went on to show a correlation between levels of insulin mRNA in the thymus and susceptibility to IDDM [14••,15••] (Table 3). Among polymorphic genetic loci that correlate with susceptibility or resistance to IDDM, one (*IDDM2*) maps to a minisatellite repeat element (a variable number of tandem repeats [VNTR] minisatellite) located a few kilobases 5′ of the human insulin gene. There are two main polymorphic allele classes, distinguishable by the length of the minisatellite element. The short version (class I) correlates with predisposition to develop IDDM, whereas the long version (class III) segregates as a resistant allele. Notably, one copy of a class III allele is dominant-protective. Remarkably, class III alleles show 2–3-fold higher levels of thymic expression than class I alleles. That is not the case in the pancreas, where if anything the levels of insulin mRNA are lower for class III alleles. Thus the correlation is between higher levels of insulin gene transcription in human thymus and resistance to development of autoimmunity, that is to say tolerance.

A second line of evidence linking thymic expression and resistance to autoimmunity comes from the RIP-transgenic

mouse systems expressing SV40 Tag or LCMV-GP/NP (Table 3). In contrast to the prototypical RIP1–Tag2 and RIP3–Tag2 mouse lines that demonstrate systemic tolerance of Tag, other lines of RIP–Tag mice are nontolerant to Tag and prone to spontaneous autoimmunity [28]. One of these lines, RIP1–Tag5, develops autoimmunity towards the islet β cells with 100% penetrance — as evidenced by leucocyte infiltration of the islets and the appearance of circulating anti-Tag autoantibodies. When assessed for thymic PAE cells expressing Tag, none could be detected using either a highly sensitive RT-PCR method or immunostaining [24••]. In contrast, the tolerant RIP–Tag mice all express Tag in thymic PAE cells [24]. Similarly, susceptibility towards autoimmunity evoked by LCMV infection varies according to thymic expression. RIP–LCMV-transgenic mice that express NP or GP in their thymi develop autoimmmunity with a long latency (1–3 months) after infection with LCMV, whereas mice that do not express detectable GP/NP in their thymi show rapid induction of autoimmune diabetes — within eight days of infection [7].

Additionally, in studies on rat and mouse models of experimental autoimmune uveoretinitis (EAU), a correlation has been observed between thymic expression of genes encoding the retinal-specific antigens arrestin and IRBP and susceptibility to EAU that is induced by those antigens [20••]. The rodent strains that express arrestin or IRBP in their thymi are relatively or completely resistant to induction of EAU by that particular antigen, whereas those strains that do not demonstrate thymic expression are highly susceptible to autoimmunity induced by these antigens.

Thus, human and rodent genetics and mouse transgenetics all suggest that thymic expression of tissue-specific genes may contribute significantly toward resistance to organ-specific autoimmunity. The data, while correlative, have profound implications. Not only can thymic expression of an organ-specific gene manifest experimental self-tolerance toward its product as revealed by immune response assays, it can apparently contribute toward a keenly important form of self-tolerance — namely the capability to resist attacking one's own cells.

Conclusion and future perspective

Expression in the thymus of genes thought to be tissue-specific is now indisputable, with the number of examples rising steadily. The examples of the myelin basic protein gene and genes of endocrine and exocrine pancreas have been bolstered by reports of genes selective for retina, brain, thyroid, placenta and liver. It is not inconceivable that genes characteristic for every organ and distinctive cell type will prove to be expressed in the thymus. As a general rule the levels of expression are low, detectable only by very sensitive RT–PCR procedures and in some cases by immunoblotting or immunohistochemistry when high-quality antibodies are available. The insulin genes, and transgenes employing their regulatory elements, have been the most extensively characterized and are found to be expressed in rare cells localized to the thymic medulla. These cells, dubbed PAE cells, express class II MHC and show cellular and antigenic characteristics of bone-marrow-derived dendritic cells. In the case of insulin-PAE cells, there appear to be 100–200 in a mouse thymus. Thymic transplantation experiments in the mouse have demonstrated, in three independent studies, that thymic expression of insulin-promoter transgenes is capable of eliciting partial self-tolerance toward the transgene product. Correlative studies in humans present a provocative argument that thymic expression levels of the insulin gene influence the predisposition to autoimmune diabetes, with higher thymic expression levels of insulin correlating with dominant-resistance genotypes. This proposition is supported by two transgenic mouse models of β cell autoimmunity — where thymic expression also correlates with resistance, whereas a lack of detectable expression is linked with susceptibility to autoimmunity. Collectively, the data support the notion that the thymus contains specialized PAE cells that serve to begin the tolerization process for rare antigens, with the specific goal of attenuating the propensity for autoimmune reactions against such antigens and against the cells that produce them.

The PAE cells of the thymic medulla are still largely mysterious and a number of questions bear mention and discussion, so as to set a framework for their further characterization.

What is the lineage of PAE cells and are there multiple cell types?

There is clear experimental evidence that medullary epithelium can tolerize developing lymphocytes [23••,29–31,32•]; moreover it is possible that cortical epithelial cells, or a specialized subset thereof, may also play a role in negative selection to abrogate T cells that are self-reactive [33]. Thus, it is attractive to consider that thymic epithelium could be quite heterogeneous, containing within it an ensemble of PAE cells expressing different tissue-specific genes (for example insulin); however, cell-fractionation protocols that enrich for bone-marrow-derived dendritic cells suggest that the insulin-PAE cells also have characteristics of dendritic cells. Analyses of other peripheral genes have given different results — the PAE cells expressing glucagon, somatostatin or pancreatic polypeptide segregate with the insulin-PAE cells but express a macrophage marker (F4/80) and not the dendritic marker (N418) seen for insulin-PAE cells. An increasing list of other PAE cells has characteristics (e.g. radio-resistance) of epithelial cells. Clarification of the issues of cell type and lineage should come, on the one hand, from additional transplantation experiments utilizing grafts of either early thymic epithelium (at embryonic day 10.5, before emigration from the bone marrow) or defined hematopoeitic progenitors and, on the other hand, from the development of transgenes expressing highly sensitive reporters that afford cell isolation and double-label immunohistochemical analysis to clarify the cellular characteristics of PAE cells. A recent report detailing thymic expression (in

rare cells of the cortex and medulla) and the incomplete tolerizing capability of an acetylcholine-receptor/β-gal transgene [34•] adds, to the growing list, a neuromuscular gene whose product is an autoantigen (in myasthenia gravis); it illustrates the potential of sensitive reporters for the characterization of thymic PAE cells. Beyond this major issue, a number of provocative questions come to mind, as noted briefly below.

Does an individual PAE cell express one, a few or many peripheral genes?

At the present time there are no clues. The observation that only a few hundred cells in thymic medulla express insulin is consistent with the possibility that one or a few genes are expressed by an individual PAE cell, while an ensemble of PAE cells encompasses many or all tissue-specific genes.

How are tissue-specific genes regulated in PAE cells of thymic medulla?

A number of issues remain to be investigated. Are there cryptic PAE enhancer elements associated with such genes? Can lessons be applied from the rare sensory cells involved in taste and smell — that express only one or a few sensory receptors, while the ensemble of the cell type expresses many [35]?

By what mechanism do PAE cells impart their effects?

The split and variable tolerance seen in thymic transplant experiments argues that PAE cells can, for some thymocyte interactions, directly induce anergy or apoptosis while allowing other autoreactive thymocytes to mature. Is a threshold of high TCR–peptide–MHC affinity the determinant for intrathymic T-cell anergy/apoptosis upon encounter with a PAE cell; or is it either the levels of peripheral antigen being expressed per cell or the abundance of specific thymic PAE cells (or a combination thereof)?

Do inadequate levels of tissue-specific genes in the thymus cause impaired self tolerance and increase susceptibility to autoimmunity?

The genetic analysis of the human *IDDM2* locus argues that thymic levels of potential autoantigens such as insulin may be one of the crucial factors affecting development of autoimmune diabetes. Beyond genetic polymorphism, perhaps somatic variation in thymic expression levels among individuals contributes; an example could be the catastrophically rapid development of IDDM in very young children, compared both to the slower development of autoimmune diabetes in older children and adults and to its absence in most people? Regarding diabetes it would be instructive (albeit logistically challenging) to investigate thymic expression levels of other autoantigen genes (including glutamic acid decarboxylase) in normal and pre-disease individuals, and to assess the nonobese diabetic (NOD) mouse and BB rat models of IDDM for autoantigen expression in their thymi. Interestingly, expression of proinsulin in cells that are positive for MHC class II (including thymus cells) in NOD-transgenic mice prevented insulinitis and diabetes (i.e. islet tolerance was established) [36]; this is consistent with the possibility that insulin gene expression in the thymi of NOD mice is usually insufficient for tolerance induction. Considering other autoimmune diseases (such as multiple sclerosis, uveoretinitis and rheumatoid arthritis) — might PAE cells be involved in resisting autoimmunity and might PAE cell dysfunction occur in the disease ontogeny? Animal models, particularly transgenic mice, should continue to shed light on these questions.

Can the PAE cells be isolated en masse and immortalized to grow in culture?

A major current limitation to their investigation is the rarity of PAE cells. Not only would it be of interest to identify particular PAE sets, for example the one that expressed insulin, it would also be valuable to find cell markers that define the PAE cell type. Efficient purification and/or cell culture would open up investigations into the cell biology and molecular biology of the PAE cell.

Could purified PAE cells be used in cell transplantation therapies to elicit tolerance and counteract or prevent autoimmunity?

If the PAE cell of thymic medulla indeed serves a generalized role in peripheral tolerance induction and if its failures contribute to autoimmunity, then perhaps PAE cell transfer into the thymus could impact autoimmune disease progression by improving the tolerization of developing thymocytes.

Acknowledgements

I wish to thank Alberto Pugliese, for many thoughtful comments and suggestions, for communicating unpublished data and for providing Figure 1; and Theresa Lopez, for insightful comments. The work mentioned from my laboratory has been supported by the US National Institutes for Health.

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This is one of two papers (with [14••]) which report that the human insulin gene is expressed in the thymus and that the levels of expression vary according to the allele class of the IDDM2 diabetes-susceptibility locus, implicating higher levels of thymic expression as being protective against autoimmune diabetes. This paper also documents the presence of proinsulin protein in human thymus and assesses (in preliminary experiments) the expression in human thymus of several other candidate autoantigens.

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This documents the expression of a nervous-system protein in thymic stroma and reports a limited tolerizing capability of thymic expression, which can be overruled by experimental immunization.

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The authors demonstrate the expression of a trophoblast-specific gene product, HLA-G, in thymus – predominantly in medullary epithelial cells.

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The authors report on the expression of two liver acute-phase genes in thymus; they document, using embryonic day 15 (E15) thymus transplantation or bone marrow reconstitution, that a human C-reactive protein transgene can transfer tolerance to the human protein if expressed in the context of E15 thymus but not via the bone marrow.

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