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Promiscuous gene expression in the thymus: The root of central tolerance

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Abstract
The thymus is a complex organ with an epithelium formed by two main cell types, the cortical thymic epithelial (cTECs) and medullary thymic epithelial cells (mTECs), referred to as stroma. Immature thymocytes arising from the bone marrow, macrophages and dendritic cells also populate the thymus. Thymocytes evolve to mature T cells featuring cell differentiation antigens (CDs), which characterize the phenotypically distinct stages, defined as double-negative (DN), double positive (DP) and single positive (SP), based on expression of the coreceptors CD4 and CD8. The thymus is therefore implicated in T cell differentiation and during development into T cells thymocytes are in close association with the stroma. Recent evidence showed that mTECs express a diverse set of genes coding for parenchymal organ specific proteins. This phenomenon has been termed promiscuous gene expression (PGE) and has led to the reconsideration of the role of the thymus in central T cell tolerance to self-antigens, which prevents autoimmunity. The evidence of PGE is causing a reanalysis in the scope of central tolerance understanding. We summarize the evidence of PGE in the thymus, focusing particularly the use of cDNA microarray technology for the broad characterization of gene expression and demarcation of PGE emergence during thymus ontogeny.

Keywords: *Autoimmunity, cDNA microarray, promiscuous gene expression, self–non-self discrimination, T cell tolerance, thymus*

Considerations on self–non-self discrimination

Self–non-self discrimination is an essential property of the immune system, which contributes to body homeostasis. The germinal idea of clonal selection theory and self-discrimination was developed by Paul Ehrlich more than 100 years ago (Ehrlich and Morgenroth 1901) and represents up to the present, the basic conceptual orientation for all immunological research.

In this regard, the direct demonstration of the clonal deletion of self-reactive lymphocytes is among the most important achievements, which has contributed to our contemporary understanding of self-tolerance in adaptive (Schwartz and Mueller 2003) and in innate (Medzhitov and Janeway 1997) immune systems (for further reading, see review of Kyewski and Derbinski 2004).

The two known pathways of immune response are characterized by the use of limited germline-encoded

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receptors for microbial components in the innate immune system or by highly diverse, somatically generated (somatic DNA rearrangements) antigen-specific T cell receptor (TCR) or immunoglobulin (Ig) receptors in adaptive immune system.

As an intellectual exercise, the heterodimeric TCR molecule (e.g. α/β TCR) could materialize the functional concept of the immune system due to their property in instantly recognizing the self major histocompatibility complex (MHC) and the non-self antigenic peptide.

Self-tolerance of the T cell repertoire is acquired during the development of immature T cells, a phenomenon dependent on the avidity of the interaction between specific TCR and self-peptide-MHC ligands. The nascent T cell repertoire in the thymus determines the diversity of self-antigens and the specificity of central self-tolerance. We agree with Kyewski and Derbinski (2004) in subsuming under central tolerance the intrathymic mechanisms, which T cells undergo in recognition of self-antigens. Accordingly, self-reactive regulatory T (T_{reg}) cells are selected in the thymus, even though these cells play their role in the periphery.

In fact, all subsets of antigen-presenting cells (APCs), such as cortical thymic epithelial cells (cTECs), medullary thymic epithelial cells (mTECs), thymic dendritic cells and macrophages play their roles in presenting unique sets of self-peptides, contributing to the diversity of self-antigens displayed in the thymus (Klein and Kyewski 2000).

However, the expression of tissue-specific antigens (TSAs) in the thymus, which represents a key feature for effecting self-representation, was only recently recognized. The evidence of thymic expression of TSAs in mice and humans, which has been referred to as promiscuous gene expression (PGE) (Jolicoeur et al. 1994; Derbinski et al. 2001; Gotter et al. 2004), reinforced the conception of central tolerance of tissue-specific self-antigens.

Prior to this evidence, the peripheral tolerance, that is, the mechanisms by which T cell selection towards TSAs occurs outside the thymus, dominated the scenario in explaining self–non-self discrimination (Alferink et al. 1999; Walker and Abbas 2002).

Promiscuous gene expression in the thymus is causing a reversal in the scope of central tolerance understanding, allowing for an unorthodox conception of the possible mechanisms of self–non-self discrimination (Kyewski et al. 2002; Gallegos and Bevan 2006).

The main implication of this heterogeneous gene expression in the thymus is associated with the maintenance of the immunological homeostasis in the body, controlling the pathogenic autoimmune reactions.

Evidence for this phenomenon was obtained using the reverse transcription-PCR (RT-PCR) method, which was biased towards antigens in autoimmune reactions, such as insulin, acetylcholine receptor or myelin basic protein. Today it is recognized that rather than selective, the set of expressed genes is as broad as possible, estimated to include up to 5–10% of all currently known mouse genes (Derbinski et al. 2001; Gotter et al. 2004; Kyewski and Derbinski 2004).

Using the microarray technology

At present, to explore the broad gene expression in the thymus, the choice strategy is the use of the microarray technology. The group of Kyewski and Derbinski (2004) from the German Cancer Research Centre in Heidelberg, Germany, has used the Affymetrics oligonucleotide microarray platform, which has allowed for extensive characterization of PGE in the mouse thymus.

In fact, large scale gene expression measurements by microarrays, in their different formats, such as, nylon membranes or glass slides have been used for more than 10 years to study the control of gene expression in the thymus, focusing mainly on thymocytes (Nguyen et al. 1995; Espanhol et al. 2004; Puthier et al. 2004; Magalhães et al. 2005; Cardoso et al. 2006).

The control of gene expression during the development of this organ has gained priority among several research teams, including our own group, allowing for the identification of candidate genes involved in thymopoiesis (Espanhol et al. 2004; Magalhães et al. 2005; Cardoso et al. 2006). A number of expressed sequence tags (ESTs) have been found that are modulated during the *in vivo* development of the thymus (Espanhol et al. 2004) and several cell-signaling genes, including those of the calcium cascade pathway, which is important for individual stages of T cell maturation and the control of anergy during thymus ontogeny (Magalhães et al. 2005).

Regarding in-lab cDNA microarray preparation for PGE research purposes in the mouse, of particular interest is the Soares thymus 2NbMT cDNA library constructed by Maria F. Bonaldo and Marcelo B. Soares of the Columbia University, New York, USA, in cooperation with Bertrand Jordan of the Centre d'Immunologie de Marseille-Luminy, France, whose researchers have made this library available at the IMAGE Consortium (http://image.llnl.gov). This is an EST normalized library, prepared from a C57Bl/6J 4 week-old male thymus and whose cDNA inserts, ranging from 0.5 to 1.5 kb in length, were cloned in the pT7T3D vector.

The library is composed of more than 25,000 resequenced clones representing most, if not the whole set of expressed genes by the mouse thymus, including those representing parenchymal and lymphoid organs. Thus, it represents a precious resource for preparing

"specialized" thymus cDNA microarrays for further use in promiscuous gene expression determinations.

The work on gene expression of fetal mouse thymus realized by our group is performed using two cDNA microarray platforms; the first version is prepared on a glass slide containing 4500 target sequences per slide, which are hybridized with fluorescent cDNA probes labeled with Cy3 or Cy5. The second version is prepared on positively charged nylon membranes containing 750 target sequences, which are hybridized with radioactive cDNA probes labeled with ^{33}P isotope.

We defined as "complex probe", the labeled cDNA originated from thymus total RNA and as "target", the cloned sequences deposited in individual spots on the microarrays.

All target cDNA clones deposited on these two versions of microarrays are from the thymus 2NbMT normalized library mentioned above, which were previously amplified by PCR in 384- or 96-well plates using vector-PCR amplification with the following primers, which recognize the cloning vector: LBP 1S GTGGAATTGTGAGCGGATACC forward and LBP 1AS GCAAGGCGATTAAGTTGG reverse.

For both versions of microarrays, a Generation III array spotter (Amersham—Molecular Dynamics—Sunnyvale, CA, USA) is used according to the manufacturer's instructions and cross-linked using an ultraviolet cross-linker.

The cDNA probes are prepared by reverse transcription using 10 μg of total RNA from fetal or adult thymus, which are labeled with Cy3 or Cy5 fluorochromes using the CyScribe post labeling kit (GE Healthcare, USA) and oligonucleotide dT_{12-18} as a primer. The 15 h period required for glass slide hybridization followed by washing is performed at 42°C in an automated slide processor (ASP, Amersham Biosciences) and the microarrays are scanned in a Generation III laser scanner (Amersham Biosciences).

The cDNA complex probes derived from fetal or adult thymus are Cy5-labeled. A Cy3-labeled cDNA pool, originated from a mix of equimolar amount of total RNA from different fetal organs (brain, liver, intestines and spleen), is used as reference in the two-color hybridizations.

The nylon cDNA microarrays are hybridized with radiolabeled ^{33}P-cDNA complex probes in a rolling oven at 65°C for 72 h and scanned in a phosphor imager storage system (Cyclone model, Packard Instruments, USA).

The characterization of each cDNA sequence is updated using the SOURCE genome data bank (http://genome-www5.stanford.edu/cgi-bin/source/sourceSearch), providing information such as DNA and protein sequences, biological and molecular functions and chromosomal location.

Preparing thymus tissue and total RNA for PGE experiments

The classic Balb-c, C57Bl/6 and (♀Balb-c × ♂C57Bl/6)F_1 mouse strains should be preferentially bred in an isolator with 0.45 μm pore sized air filtered. To obtain timed pregnancies of Balb-c, C57Bl/6 and their hybrids at the developmental phase when TRBV8.1 V(D)J recombination occurs, the mice are mated and the day when the vaginal plug is observed at 7:00 am is considered to be day zero of gestation post coitum (p.c.). The pregnant mice are sacrificed preferentially by CO_2 inhalation and the fetuses collected by surgery of the uterus. The p.c. age of fetuses should be confirmed by observing the morphological characteristics of each developmental phase (Rugh 1968).

The onset of TCR beta V(D)J recombination (TRBV8.1-BD2.1) during the *in vivo* fetal development of the thymus of Balb-c, C57Bl/6 and their hybrids is taken on the basis of previously published data. Fetal thymus should be obtained at the emergence of V(D)J recombination, that is, at 15–16 days p.c. for Balb-c and 14–15 days p.c. for C57Bl/6 and (♀Balb-c × ♂C57Bl/6)F_1 (Macedo et al. 1999; Magalhães et al. 2005).

The thymi should be removed from the fetuses preferable under a stereomicroscope and the epithelial cells preparation enriched on Percoll® gradient centrifugation or purified by cell sorting, which are immediately processed for total RNA extraction.

In the microarray experiments, it is extremely important to use only undegraded and DNA-, protein- and phenol-free RNA preparations as verified by classic agarose gel electrophoresis stained with ethidium bromide and ultraviolet spectrophotometry, respectively.

Analysing microarray data by statistical significance algorithm

The gene expression data obtained from microarray experiments should be analysed by a mathematical/-statistical algorithm. In PGE projects, we are using the significance analysis of microarrays method (SAM, available online at http://www-stat.stanford.edu/~tibs/SAM/index.html) to analyse the significant variations in gene expression (Tusher et al. 2001). This method is based on *t*-test statistics, specially modified for high throughput analysis. We propose discussing only those genes which present a fold change expression ≥ 1 (induced) and a false discovery rate (FDR) max. 0.05 (Table I, available online at http://rge.fmrp.usp.br/passos/review/PGE/table1).

To analyse the significant variations in the gene expression of developing thymus, RNA samples are extracted on fixed days p.c. are compared with RNA from subsequent days of gestation.

Table I. Genes differentially and significantly induced in the thymus of C57Bl/6 and (♀Balb-c × ♂C57Bl/6)F_1 as detected by the SAM program. Fold change ≥ 1 and FDR, false discovery rate. Magalhães et al. (2006) promiscuous gene expression in the thymus.

Gene name	GenBank accession	Chromosomes	Predominant expression	Molecular/biological function
C57Bl/6 strain (fold change = 1.5 and FDR ~ 0.05)				
Hexosaminidase B (Hexb)	NM_010422	13	Adipose tissue, bone, amygdale, frontal cortex, trigeminal, cerebral cortex, dorsal root ganglia, dorsal striat um, hippocampus, hypothalamus, olfactory bulb, spinal cord'lower, spinal cord upper, substantia nigra, blato-cystis, placenta, small intestine, B220+B-cells, lymph-node, vomeronasal organ, salivary gland, pituitary, snout epidermis, thymus, thyroid, trachea, bladder, kidney	Metabolism
Bromodomain containing 4 (Brd4)	NM_020508	17	Adipose tissue, bone, bonemarrow, amygdala, preoptic, hippocampus, spinal cord'lower, embryo day 10.5, embryo day 6.5, embryo day 7.5, embryo day 8.5, embryo day 9.5, ovary, prostate, umbilical cord, uterus, heart, small intestine, B220+B-cells, CD4+ Tcells, CD8+ T cells, lung, lymphnode, pancreas, digits, snout epidermis, thymus, trachea	Required maternally for proper expression of other homeotic genes involved in pattern formation, such as ubx
Stimulated by retinoic acid 13 (Stra13)	NM_016665	11E2	Adipose tissue, adrenalgland, bone, main olfactory epithelium, embryo day 6.5, embryo day 9.5, fertilized egg, ovary, prostate, testis, umbilical cord, uterus, oocyte, large intestine, B220+B-cells, CD4+ T cells, CD8+ T cells, liver, lung, vomeronasal organ, salivary gland, tongue, pituitary, digits, snout epidermis, spleen, thymus, trachea, kidney	–
(♀Balb-c × ♂C57Bl/6)F_1 (fold change = 1.0 and FDR ~ 0.0012)				
Ets2 repressor factor (Erf)	NM_010155	7	Brown fat, bonemarrow, dorsal striatum, hippocampus, blastocysts, embryo day 10.5, embryo day 6.5, embryo day 7.5, embryo day 8.5, embryo day 9.5, fertilized egg, mammary gland (lact), ovary, placenta, heart, small intestine, liver, skeletal muscle, salivary gland, pancreas, spleen, stomach, thyroid, bladder	Transcriptional repressor (by similarity)
RAB2, member RAS oncogene family (Rab2)	NM_021518	4A1	Adrenal gland, amygdala, frontal cortex, preoptic, trigeminal, cerebellum, cerebral cortex, dorsal root ganglia, dorsal striatum, hippocampus, hypothalamus, main olfactory ephitelium, olfactory bulb, spinal cord lower, spinal cord upper, substantia nigra, fertilized egg, ovary, prostate, oocyte, large intestine, lung, skeletal muscle, vomeronasal organ, pituitary, digits, epidermis, bladder, kidney, retina	Required for protein transport from the endoplasmic reticulum to the golgi complex (by similarity)

Table I – *Continued*

Gene name	GenBank accession	Chromosomes	Predominant expression	Molecular/biological function
Membrane associated DNA binding protein (Mnab)	XM_130233	2	Adrenal gland, amygdala, frontal cortex, preoptic, trigeminal, cerebellum, cerebral cortex, dorsal root ganglia, dorsal striatum, hippocampus, hypothalamus, main olfactory ephitelium, olfactory bulb, spinal cord lower, substantia nigra, embryo day 10.5, ovary, prostate, umbilical cord, lung, skeletal muscle, vomeronasal organ, tongue, pituitary, digits, epidermis, thymus, trachea, bladder, retina	Nucleic acid binding
RAD51-like 1 (S. cerevisiae) (Rad51l1)	NM_009014	12 C3	Brown fat, bonemarrow, dorsal striatum, embryo day 9.5, fertilized egg, mammary gland (lact), ovary, placenta, testis, uterus, B220+B-cells, CD4+ Tcells, salivary gland, pancreas, spleen, stomach, thyroid, trachea	DNA repair (by similarity)
Small chemokine (C–C motif) ligand 11 (Ccl11)	NM_011330	11	Brown fat, adipose tissue, trigeminal, ovary, prostate, uterus, large intestine, small intestine, lymphnode, skeletal muscle, tongue, digits, epidermis, snout epidermis, stomach, thymus, thyroid, trachea, bladder	Immune response by eosinophils
DEAD (Asp-Glu-Ala-Asp) box polypeptide 21 (Ddx21)	NM_019553	10	Adipose tissue, adrenal gland, bone, blastocysts, embryo day 10.5, embryo day 6.5, embryo day 7.5, embryo day 8.5, embryo day 9.5, mammary gland (lact), ovary, prostate, umbilical cord, uterus, larger intestine, B220+B-cells, CD4+ Tcells, CD8+ Tcells, lung, lymphnode, salivary gland, tongue, digits, epidermis, spleen, thymus, trachea, bladder	Nucleic acid binding RNA helicase/foldase (by similarity)
Sodium channel modifier 1 (Scnm1)	NM_027013	3	Brown fat, frontal cortex, preoptic, trigeminal, hippocampus, hypothalamus, main olfactory ephitelium, spinal cord lower, substantia nigra, blastocysts, embryo day 10.5, embryo day 6.5, embryo day 7.5, embryo day 9.5, ovary, testis, heart, B220+B-cells, CD4+ Tcells, CD8+ Tcells, tongue, pituitary, digits, snout epidermis, thymus, trachea	Ion channel activity and RNA splicing
Sialophorin (Spn)	NM_009259	7	Bone, bonemarrow, embryo day 10.5, embryo day 6.5, embryo day 7.5, embryo day 9.5, placenta, B220 + B-cells, CD4+ Tcells, CD8+ Tcells, lung, lymphnode, spleen, thymus, trachea	Negative regulatory role in adaptive immune response (by similarity)
Adenosine deaminase (Ada)	NM_007398	2	Placenta, small intestine, tongue, thymus, trachea	Immune response, nucleotide metabolism
Ecotropic viral integration site 2[a] (Evi2a)	NM_010161	11	Brown fat,bone, bonemarrow, dorsal striatum, hippocampus, spinal cord upper, blastocysts, embryo day 10.5, embryo day 6.5, embryo day 7.5, embryo day 9.5, mammary gland (lact), oocyte, heart, CD8+ Tcells, liver, lymphnode, pancreas, epidermis, spleen, stomach, thymus, thyroid, trachea	–

Table I – *Continued*

Gene name	GenBank accession	Chromosomes	Predominant expression	Molecular/biological function
B-cell leukemia/lymphoma 6 (Bcl6)	NM_009744	16	Adipose tissue, adrenal gland, bone, cerebellum, hippocampus, main olfactory ephitelium, olfactory bulb, spinal cord lower, spinal cord upper, ovary, prostate, uterus, B220+B-cells, lymphnode, skeletal muscle, salivary gland, digits, epidermis, snout epidermis, thymus, trachea, kidney, retina	Transcription factor
Interleukin 16 (Il16)	NM_010551	7	Adipose tissue, cerebellum, B220+B-cells, CD4+ Tcells, CD8+ Tcells, lymphnode, spleen, thymus, trachea	Cytokine activity, immune cell chemotaxis
Chemokine (C–X–C motif) ligand 4 (Cxcl4)	NM_019932	5 E1	Adipose tissue, bone, bonemarrow, trigeminal, ovary, umbilical cord, heart, lung, skeletal muscle, vomeronasal organ, tongue, digits, epidermis, snout epidermis, spleen, trachea, bladder	Platelet factor 4, is released during platelet aggregation
Microtubule-associated protein, RP/EB family, member 2 (Mapre2)	NM_153058	18 A2	Brown fat, bonemarrow, dorsal striatum, blastocysts, embryo day 10.5, embryo day 6.5, embryo day 7.5, embryo day 9.5, mammary gland (lact), placenta, prostate, heart, small intestine, liver, lung, lymphnode, skeletal muscle, salivary gland, pancreas, spleen, stomach, thyroid, bladder	Microtubule binding and protein binding
WAP, follistatin/kazal, immunoglobulin, kunitz and netrin domain containing 2 (Wfikkn2)	NM_181819	11D	Adipose tissue, prostate, umbilical cord, uterus, CD4+ Tcells, CD8+ Tcellslung, lymphnode, longue, digits, epidermis, thymus	Alpha-1-microglobulin occurs in many physiological fluids including plasma, urine, and cerebrospinal fluid (by similarity)
Signal transducer and activator of transcription 1 (Stat1)	NM_009283	1	Adipose tissue, adrenal gland, bone, bonemarrow, trigeminal, dorsal root ganglia, ovary, uterus, B220+B-cells, CD4+ Tcells, CD8+ Tcells, lung, lymphnode, pancreas, spleen, thymus, thyroid, trachea	Transcription factor
C-type lectin domain family 1, member b (Clec1b) (Clec2)	NM_019985	6F3	Bone, bonemarrow, embryo day 10.5, placenta, liver, lymphnode, pancreas, spleen, trachea	Cell surface receptor linked signal transduction
Exportin, tRNA (nuclear export receptor for tRNAs) (Xpot)	XM_125902	10D3	Adrenal gland, amygdale, frontal cortex, preoptic, cerebellum, cerebral cortex, dorsal root ganglia, hippocampus, hypothalamus, olfactory bulb, spinal cord lower, spinal cord upper, substantia nigra, blastocysts, embryo day 10.5, embryo day 6.5, embryo day 7.5, embryo day 9.5, mammary gland (lact), ovary, prostate, umbilical cord, uterus, lung, longue, digits, thymus, trachea	Mediates nuclear export of all tRNAs (by similarity)
Ubiquitin-activating enzyme E1, Chr X (Ube1x)	NM_009457	X	Adrenal gland, preoptic, trigeminal, cerebellum, cerebral cortex, dorsal root ganglia, olfactory bulb, substantia nigra, blastocysts, embryo day 10.5, embryo day 6.5, embryo day 9.5, fertilized egg, ovary, prostate, uterus, oocyte, CD4+ Tcells, longue, digits, snout epidermis, thymus, trachea	ATP binding

Table I – *Continued*

Gene name	GenBank accession	Chromosomes	Predominant expression	Molecular/biological function
Activating transcription factor 4 (Atf4)	NM_009716	15	Adrenal gland, cerebellum, main olfactory epithelium, blastocysts, embryo day 6.5, embryo day 7.5, embryo day 9.5, placenta, umbilical cord, uterus, oocyte, large intestine, B220+B-cells, CD4+ Tcells, lung, skeletal muscle, vomeronasal organ, salivary gland, tongue, pituitary, digits, snout epidermis, thymus, trachea, retina	Transcription factor
Zinc finger protein 131 (Zfp131)	NM_028245	13	Adipose tissue, adrenal gland, bone, bonemarrow, amygdale, frontal cortex, cerebellum, embryo day 9.5, fertilized egg, ovary, uterus, oocyte, large intestine, B220+B-cells, CD4+ Tcells, CD8+ Tcells, lung, lymphnode, skeletal muscle, digits, thymus, trachea	May be involved in transcriptional regulation
RAB14, member RAS oncogene (Rab14)	NM_026697	2B	Adipose tissue, bone, bonemarrow, preoptic, blastocysts, ovary, umblical cord, lymphnode, longue, digits, epidermis, snout epidermis, thymus*, trachea	Required for protein transport in the secretory pathway
Synaptophysin-like protein (Sypl)	NM_198710	12	Adipose tissue, adrenalgland, bone, main olfactory epithelium, spinal cord'lower, spinal cord upper, fertilized egg, ovary, placenta, prostate, uterus, oocyte, heart, large intestine, small intestine, B220+ B cells, lung, skeletal muscle, vomeronasal organ, salivary gland, tongue, digits, epidermis, snout epidermis, stomach, trachea, bladder, kidney, retina	Transport, transporter activity
WD repeat and SOCS box-containing 2 (Wsb2)	NM_021539	5F	Adrenal gland, amygdale, frontal cortex, preoptic, trigeminal, cerebellum, cerebral cortex, dorsal root ganglia, dorsal striatum, hippocampus, hypothalamus, main olfactory ephitelium, olfactory bulb, spinal cord lower, spinal cord upper, substantia nigra, ovary, placenta, umbilical cord, uterus, large intestine, lymphnode, skeletal muscle, vomeronasal organ, pancreas, pituitary, spleen, kidney	Intracellular signaling cascade
POU domain, class 2, associating factor 1 (Pou2af1)	NM_011136	9 A5.3	Adipose tissue, bone, bonemarrow, B220+B-cells, CD4+ Tcells, CD8+ Tcells, lymphnode, spleen, trachea	DNA binding, regulation of transcription
Transformed mouse 3T3 cell double minute 2 (Mdm2)	NM_010786	10	Adrenal gland, amygdale, preoptic, spinal cord lower, substantia nigra, blastocysts, embryo day 6.5, embryo day 8.5, embryo day 9.5, fertilized egg, placenta, testis, oocyte, B220+B-cells, CD4+ Tcells, CD8+ Tcells, lung, lymphnode, skeletal muscle, vomeronasal organ, thymus, trachea	Cell growth and/or maintenance
Interleukin 4 (Il4)	NM_021283	11	Brown fat, bonemarrow, dorsal striatum, hippocampus, embryo day 10.5, embryo day 7.5, embryo day 9.5, fertilized egg, mammary gland (lact), placenta, testis, heart, large intestine, small intestine, liver, skeletal muscle, salivary gland, longue, pancreas, spleen, stomach, thyroid, bladder, kidney	Participates in at least several b-cell activation processes as well as of other cell types

Table I – *Continued*

Gene name	GenBank accession	Chromosomes	Predominant expression	Molecular/biological function
Protein O-fucosyltransferase 2 (Pofut2)	NM_030262	10C1	Brown fat, Adipose tissue, adrenal gland, preoptic, cerebellum, main olfactory epithelium, embryo day 6.5, embryo day 7.5, embryo day 8.5, embryo day 9.5, fertilized egg, ovary, placenta, testis, umbilical cord, uterus, oocyte, lung, vomeronasal organ, pituitary, snout epidermis	Carbohydrate metabolism
CD24a antigen (Cd24a)	NM_009846	10	Adipose tissue, bone, bonemarrow, trigeminal, dorsal root ganglia, main olfactory epithelium, embryo day 10.5, embryo day 8.5, embryo day 9.5, mammary gland (lact), ovary, prostate, uterus, large intestine, B220+B-cells, lung, skeletal muscle, vomeronasal organ, salivary gland, longue, digits, spleen, stomach, thymus, thyroid, trachea, kidney, retina	May have a pivotal role in cell differentiation
Transcription factor E2a (Tcfe2a)	NM_011548	10	Adipose tissue, adrenal gland, bone, bonemarrow, cerebellum, main olfactory epithelium, embryo day 10.5, embryo day 6.5, embryo day 7.5, embryo day 8.5, embryo day 9.5, fertilized egg, ovary, umbilical cord, uterus, oocyte, B220+B-cells, CD4+ Tcells, CD8+ Tcells, lymphnode, pituitary, digits, epidermis, snout epidermis, spleen, thymus, trachea, bladder	Transcription factor
Interferon-induced protein with tetratricopeptide repeats 2 (Ifit2)	NM_008332	19C1	Adipose tissue, adrenal gland, bone, retina, bonemarrow, trigeminal, dorsal root ganglia, hypothalamus, main olfactory epithelium, olfactory bulb, spinal cord lower, substantia nigra, ovary, placenta, prostate, uterus, heart, large intestine, B220+B-cells, CD4+ Tcells, CD8+ Tcells, lung, lymphnode, vomeronasal organ, epidermis, spleen, thymus, trachea, kidney	Immune response
Cut-like 1 (Drosophila) (Cutil)	NM_009986	5	Brown fat, adrenalgland, bonemarrow, frontal cortex, preoptic, cerebellum, dorsal striatum, main olfactory epithelium, olfactory bulb, spinal cord'lower, substantia nigra, embryo day 10.5, embryo day 7.5, embryo day 8.5, embryo day 9.5, fertilized egg, mammary gland (lact), placenta, umbilical cord, uterus, heart, large intestine, small intestine, liver, lung, skeletal muscle, omeronasal organ, salivary gland, tongue, pancreas, digits, epidermis, snout epidermis, spleen, stomach, thymus, thyroid, bladder, kidney, retina	Probably has a broad role in mammalian development as a repressor of developmentally regulated gene expression
Histocompatibility 2, complement component factor B (H2-Bf)	NM_008198	17	Adipose tissue, ovary, uterus, large intestine, small intestine, liver, lymphnode, digits, epidermis, snout epidermis, spleen, trachea, kidney	Factor B which is part of the alternate pathway of the complement system
Fanconi anemia, complementation group G (Fancg)	NM_053081	4B1	Bone, bonemarrow, fertilized egg, testis, oocyte, B220+B-cells, CD4+ Tcells, CD8+ Tcells, salivary gland, longue, digits, snout epidermis, thymus, trachea	Binding,DNA repair, response to DNA damage stimulus, response to radiation, spermatid development

Table I – *Continued*

Gene name	GenBank accession	Chromosomes	Predominant expression	Molecular/biological function
Ras homolog gene family, member A (Rhoa)	NM_016802	9	Adipose tissue, adrenal gland, bone, dorsal root ganglia, main olfactory epithelium, embryo day 10.5, embryo day 6.5, embryo day 7.5, embryo day 8.5, embryo day 9.5, ovary, prostate, umbilical cord, uterus, heart, large intestine, small intestine, B220+B-cells, CD4+ Tcells, CD8+ Tcells, lung, vomeronasal organ, digits, snout epidermis, stomach, thymus, trachea, bladder, kidney	Regulates a signal transduction pathway linking plasma membrane receptors to the assembly of focal adhesions and actin stress fibers
Interferon-induced protein with tetratricopeptide repeats 1 (Ifit1)	NM_008331	19C1	Brown fat, Adipose tissue, adrenal gland, bone, bonemarrow, trigeminal, dorsal root ganglia, main olfactory epithelium, ovary, placenta, prostate, uterus, heart, large intestine, small intestine, CD4+ Tcells, CD8+ Tcells, liver, lung, lymphnode, vomeronasal organ, longue, pancreas, epidermis, spleen, thymus, trachea, bladder, retina	Immune response
Growth factor receptor bound protein 2 (Grb2)	NM_008163	11	Bone, bonemarrow, preoptic, cerebellum, cerebral cortex, dorsal root ganglia, dorsal striatum, hippocampus, hypothalamus, olfactory bulb, spinal cord'lower, substantia nigra, embryo day 10.5, embryo day 6.5, embryo day 7.5, embryo day 8.5, embryo day 9.5, heart, B220+ B cells, CD4+ T cells, CD8+ T cells, lymphnode, tongue, epidermis, snout epidermis	MAPKKK cascade, protein binding, Ras protein signal transduction, SH3/SH2 adaptor activity
Histamine receptor H 3 (Hrh3)	NM_133849	2H4	Bone, amygdale, frontal cortex, preoptic, trigeminal, cerebellum, córtex cerebral, dorsal striatum, hippocampus, hypothalamus, olfactory bulb, spinal cord lower, spinal cord upper, substantia nigra embryo day 7.5, embryo day 9.5, placenta, lung, salivary gland, pancreas, pituitary, spleen, stomach, thyroid, retina	The H3 subclass of histamine receptors could mediate the histamine signals in CNS and peripheral nervous system (by similarity)
Tumor necrosis factor receptor superfamily, member 4 (Tnfrsf4)	NM_009452	1	Adipose tissue, main olfactory epithelium, testis, heart, small intestine, B220+B-cells, CD4+ Tcells, lymphnode, skeletal muscle, epidermis, snout epidermis, trachea, kidney	Cellular defense response,inflammatory response
Wiskott-Aldrich syndrome protein interacting protein (Waspip)	NM_153138	2	Adipose tissue, bone, bonemarrow, dorsal root ganglia, spinal cord lower, blastocysts, embryo day 10.5, mammary gland (lact), umbilical cord, uterus, heart, B220+B-cells, CD4+ Tcells, CD8+ Tcells, lung, lymphnode, vomeronasal organ, longue, digits, snout epidermis, spleen, thymus, trachea, bladder	Actin binding, actin filament-based movement

Table I – *Continued*

Gene name	GenBank accession	Chromosomes	Predominant expression	Molecular/biological function
BCL2/adenovirus E1B 19 kDa-interacting protein 1, NIP3 (Bnip3)	NM_009760	7F5	Brown fat, Adipose tissue, adrenal gland, frontal cortex, trigeminal, cerebral cortex, dorsal root ganglia, hypothalamus, spinal cord lower, spinal cord upper, substantia nigra, blastocysts, embryo day 6.5, embryo day 8.5, ferlized egg, ovary, placenta, prostate, umbilical cord, oocyte, heart, liver, skeletal muscle, vomeronasal organ, longue, pituitary, epidermis, trachea, kidney	Binds to the adenovirus e1b 19 kda protein or to bcl-2. may play a role in repartitioning calcium between the two major intracellular calcium stores in association with the 19 kda or bcl-2 proteins
PTK2 protein tyrosine kinase 2 beta (Ptk2b)	NM_172498	14	Adipose tissue, bone, bonemarrow, amygdale, frontal cortex, cerebral cortex, dorsal striatum, hippocampus, olfactory bulb, large intestine, B220+B-cells, CD4+ Tcells, CD8+ Tcells, lymphnode, spleen, thymus, trachea	Involved in calcium induced regulation of ion channel and activation of the map kinase signaling pathway
SMT3 suppressor of mif two 3 homolog 3 (yeast) (Sumo3)	NM_019929	10	Bone, amygdale, frontal cortex, preoptic, cerebellum, hypothalamus, main olfactory epithelium, olfactory bulb, spinal cord lower, substantia nigra, , blastocysts, embryo day 6.5, embryo day 8.5, embryo day 9.5, embryo day 10.5 ferlized egg, ovary, prostate, umbilical cord, uterus, oocyte, vomeronasal organ, pituitary, digits, snout epidermis, thymus, trachea, retina	Protein modification, ubiquitin cycle
Tumor necrosis factor receptor superfamily, member 13b (Tnfrsf13b)	NM_021349	11B2	Adipose tissue, bone, bonemarrow, B220+B-cells, CD4+ Tcells, CD8+ Tcells, lung, lymphnode, spleen, thymus, trachea	B cell homeostasis, immune response, negative regulation of B cell proliferation
Non-catalytic region of tyrosine kinase adaptor protein 2 (Nck2)	NM_010879	1C1	Adipose tissue, adrenal gland, cerebral cortex, hippocampus, main olfactory epithelium, olfactory bulb, blastocysts, embryo day 9.5, embryo day 10.5, ovary, placenta, prostate, umbilical cord, uterus, heart, large intestine, small intestine, CD4+ Tcells, CD8+ Tcells, lung, vomeronasal organ, tongue, snout epidermis, stomach, thymus, trachea, bladder	Actin filament organization, cell migration, epidermal growth factor receptor signaling pathway
T-cell receptor gamma, variable 4 (Tcrg-V4)	Z12299.1	13	Brown fat, Adipose tissue, bonemarrow, dorsal striatum, heart, large intestine, small intestine, CD4+ Tcells, CD8+ Tcells, liver, lymphnode, epidermis, spleen, thymus, trachea	Cellular defense response
F-box protein 45 (Fbxo45)	NM_173439	16B2	Adipose tissue, adrenal gland, bone, amygdala, frontal cortex trigeminal, cerebral cortex, cerebellum, dorsal root ganglia, hippocampus, main olfactory epithelium, olfactory bulb, spinal cord lower, substantia nigra, blastocysts, embryo day 9.5, embryo day 10.5, ovary, heart, skeletal muscle vomeronasal organ, tongue, pituitary, digits, epidermis, snout epidermis spleen, thymus, trachea, retina	Ubiquitin cycle

Table I – *Continued*

Gene name	GenBank accession	Chromosomes	Predominant expression	Molecular/biological function
Protein kinase, interferon inducible double stranded RNA dependent activator (Prkra)	NM_011871	2C3	Adipose tissue, adrenal gland, bone, preoptic, trigeminal, dorsal root ganglia, hypotalamus, spinal cord upper, spinal cord lower, substantia nigra, embryo day 7.5, embryo day 8.5, embryo day 9.5, embryo day 10.5, ovary, testis, umbilical cord, uterus, heart, large intestine, small intestine, liver, lung, pituitary, stomach, trachea, bladder, kidney, retina	Double-stranded RNA binding, kinase activity, protein amino acid phosphorylation
Genetic suppressor element 1 (Gse1)	NM_198671	8E1	Bone, bonemarrow, amygdale, preoptic, cerebellum, cerebral cortex, dorsal striatum, hippocampus, hypotalamus, main olfactory epithelium, olfactory bulb, spinal cord lower, substantia nigra, blastocysts, embryo day 10.5, embryo day embryo day 9.5, prostate, oocyte, large intestine, small intestine, B220+B-cells, CD4+ Tcells, lung, lymphnode, pituitary, digits, snout epidermis, thymus, bladder, retina	–
Janus kinase 1 (Jak1)	NM_146145	4	Brown fat, Adipose tissue, bone, amygdale, frontal cortex, trigeminal, cerebellum, dorsal striatum, hypotalamus, spinal cord lower, embryo day 7.5, fertilized egg, mammary gland, placenta, uterus, oocyte, heart, B220+B-cells, liver, lymphnode, salivary gland, pancreas, digits, epidermis, spleen, thymus, thyroid	Tyrosine kinase of the non-receptor type, involved in the ifn-alpha/beta/gamma signal pathway
Transportin 2 (importin 3, karyopherin beta 2b) (Tnpo2)	NM_145390	8C2	Amygdale, frontal cortex, preoptic, trigeminal, cerebellum, cerebral cortex, dorsal root ganglia, dorsal striatum, hippocampus, hypotalamus, main olfactory epithelium, olfactory bulb, spinal cord upper, spinal cord lower, substantia nigra, blastocysts, embryo day 6.5, embryo day 7.5, embryo day 9.5, ovary, prostate, vomeronasal organ, tongue, digits, snout epidermis, thymus	Required for import of mRNA binding proteins
v-rel reticuloendotheliosis viral oncogene homolog A (avian) (Rela)	NM_009045	19	Adipose tissue, adrenal gland, bone, cerebellum, main olfactory epithelium, fertilized egg, ovary, placenta, prostate, testis, umbilical cord, uterus, oocyte, B220+B-cells, CD4+ Tcells, CD8+ Tcells, lung, lymphnode, vomeronasal organ, tongue, pituitary, digits, epidermis, snout epidermis, spleen, thymus, trachea, bladder, retina	Activation of NF-kappaB transcription factor
Pyruvate dehydrogenase kinase, isoenzyme 2 (Pdk2)	NM_133667	11	Brown fat, Adipose tissue, amygdala, frontal cortex, preoptic, cerebellum, cerebral cortex, hippocampus, hypotalamus, main olfactory epithelium, olfactory bulb, spinal cord upper, spinal cord lower, substantia nigra, prostate, testis, heart, large intestine, small intestine, lung, skeletal muscle, vomeronasal organ, tongue, epidermis, snout epidermis, stomach, thymus, thyroid, kidney	Regulation of glucose metabolism

Table I – *Continued*

Gene name	GenBank accession	Chromosomes	Predominant expression	Molecular/biological function
Toll-like receptor 4 (Tlr4)	NM_021297	4	Brown fat, Adipose tissue, bone, bonemarrow, main olfactory epithelium, mammary gland, prostate, umbilical cord, uterus, heart, large intestine, B220+B-cells, liver, lung, lymphnode, skeletal muscle, vomeronasal organ, salivary gland, tongue, pancreas, digits, epidermis, spleen, stomach, thyroid, trachea, bladder	Activation of NF-kappaB-inducing kinase, catalytic activity, I-kappaB kinase/NF-kappaB cascade, immune response, inflammatory response
Zinc finger protein 143 (Zfp143)	NM_009281	7	Brown fat, Adipose tissue, bone, bonemarrow, cerebellum, main olfactory epithelium, embryo day 10.5, embryo day 6.5, embryo day 7.5, embryo day 8.5, embryo day 9.5, fertilized egg, ovary, testis, umbilical cord, uterus, oocyte, heart, B220+B-cells, CD4+ Tcells, CD8+ Tcells, lymphnode, skeletal muscle, vomeronasal organ, salivary gland, digits, snout epidermis, spleen, thymus, thyroid, trachea	Transcriptional activator. binds to the sph motif of small nuclear rna (snRNA) gene promoters
Interleukin 2 receptor, gamma chain (Il2rg)	NM_013563	X	Adipose tissue, bone, bonemarrow, embryo day 6.5, embryo day 7.5, heart, B220+B-cells, CD4+ Tcells, CD8+ Tcells, lung, lymphnode, skeletal muscle, epidermis, spleen, thymus, trachea	Cell surface receptor linked signal transduction
UDP-GlcNAc:betaGal beta-1,3-*N*-acetyl-glucosaminyltransferase 1 (B3gnt1)	NM_016888	11	Adipose tissue, adrenal gland, bone, amygdale, frontal cortex, trigeminal, dorsal root ganglia, dorsal striatum, hypotalamus, main olfactory epithelium, embryo day 6.5, fertilized egg, ovary, prostate, uterus, oocyte, large intestine, small intestine, B220+B-cells, CD4+ Tcells, CD8+ Tcells, lung, lymphnode, vomeronasal organ, pituitary, digits, snout epidermis, stomach, thymus, trachea, bladder, kidney	Axon guidance, galactosyltransferase activity, manganese ion binding, protein amino acid glycosylation, sensory perception of smell
Splicing factor 3b, subunit 1 (Sf3b1)	NM_031179	1	Adipose tissue, adrenal gland, bonemarrow, amygdale, frontal cortex, preoptic, trigeminal, cerebellum, cerebral cortex, dorsal striatum, hippocampus, hypotalamus, main olfactory epithelium, olfactory bulb, spinal cord upper, spinal cord lower, substantia nigra, embryo day 10.5, embryo day 6.5, embryo day 8.5, embryo day 9.5, fertilized egg, ovary, umbilical cord, uterus, oocyte, heart, B220+B-cells, CD4+ Tcells, lung, pituitary, thymus, trachea, bladder, kidney, retina	Subunit of the splicing factor sf3b required for "a" complex assembly formed by the stable binding of u2 snrnp to the branchpoint sequence (bps) in pre-mRNA
Lymphoid enhancer binding factor 1 (Lef1)	NM_010703	3	Brown fat, bonemarrow, blastocysts, embryo day 10.5, embryo day 6.5, embryo day 7.5, embryo day 8.5, embryo day 9.5, fertilized egg, mammary gland, placenta, prostate, testis, umbilical cord, heart, small intestine, B220+B-cells, CD4+ Tcells, CD8+ Tcells, liver, lymphnode, skeletal muscle, salivary gland, pancreas, spleen, stomach, thymus, thyroid, bladder, kidney	Transcriptional activator

Table I – *Continued*

Gene name	GenBank accession	Chromosomes	Predominant expression	Molecular/biological function
Solute carrier family 12, member 6 (Slc12a6)	NM_133649	2E3	Brown fat, bonemarrow, dorsal striatum, hippocampus, hypotalamus, olfactory bulb, substantia nigra, blastocysts, embryo day 10.5, embryo day 6.5, embryo day 7.5, embryo day 8.5, embryo day 9.5, fertilized egg, mammary gland, placenta, testis, oocyte, heart, large intestine, small intestine, B220+B-cells, CD4+ Tcells, liver, lymphnode, skeletal muscle, salivary gland, pancreas, epidermis, spleen, stomach, thymus, thyroid, bladder, kidney	Amino acid transport
Angio-associated migratory protein (Aamp)	NM_146110	1C4	Adipose tissue, adrenal gland, amygdale, preoptic, cerebellum, cerebral cortex, dorsal root ganglia, hippocampus, hypotalamus, main olfactory epithelium, olfactory bulb, spinal cord lower, substantia nigra, embryo day 6.5, ovary, placenta, prostate, testis, uterus, B220+B-cells, CD4+ Tcells, CD8+ Tcells, lymphnode, vomeronasal organ, pituitary, digits, snout epidermis, thymus, bladder, kidney	–
Nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1 (Nfact1)	NM_016791	18	Brown fat, Adipose tissue, bone, bonemarrow, main olfactory epithelium, fertilized egg, umbilical cord, B220+B-cells, CD4+ Tcells, CD8+ Tcells, lung, lymphnode, skeletal muscle, vomeronasal organ, salivary gland, tongue, pancreas, digits, epidermis, snout epidermis, spleen, stomach, thymus, thyroid, trachea	Plays a role in the inducible expression of cytokine genes in t cells (by similarity)
Actin-binding LIM protein 1 (Ablim1)	NM_178688	19	Brown fat, Adipose tissue, amygdale, preoptic, trigeminal, cerebellum, dorsal root ganglia, olfactory bulb, spinal cord lower, substantia nigra, ovary, umbilical cord, heart, large intestine, B220+B-cells, CD4+ Tcells, CD8+ Tcells, lung, lymphnode, vomeronasal organ, tongue, digits, epidermis, snout epidermis, spleen, stomach, thymus, thyroid, trachea, retina	Actin binding, axon guidance, cytoskeleton organization and biogenesis, metal ion binding, zinc ion binding
DNA methyltransferase 3A (Dnmt3a)	NM_153743	12 A2–A3	Adipose tissue, bonemarrow, amygdale, preoptic, cerebellum, cerebral cortex, dorsal striatum, hippocampus, hypotalamus, main olfactory epithelium, olfactory bulb, spinal cord upper, spinal cord lower, substantia nigra, embryo day 10.5, ovary, placenta, umbilical cord, uterus, heart, lymphnode, skeletal muscle, vomeronasal organ, pancreas, pituitary, snout epidermis, spleen, thymus, thyroid, retina	Required for genome wide de novo methylation and is essential for development

Table I – *Continued*

Gene name	GenBank accession	Chromosomes	Predominant expression	Molecular/biological function
Early growth response 1 (Egr1)	NM_007913	18	Adipose tissue, adrenal gland, amygdale, frontal cortex, preoptic, cerebellum, cerebral cortex, dorsal root ganglia, hippocampus, hypotalamus, olfactory bulb, spinal cord lower, substancia nigra, ovary, heart, small intestine, B220+B-cells, CD4+ Tcells, CD8+ Tcells, lung, lymphnode, skeletal muscle, tongue, pituitary, digits, epidermis, snout epidermis, stomach, thymus, trachea	Transcriptional regulator
Deoxyhypusine synthase (Dhps)	NM_201408	8C2	Bonemarrow, amygdale, frontal cortex, preoptic, trigeminal, cerebellum, cerebral cortex, dorsal root ganglia, dorsal striatum, hippocampus, hypotalamus, olfactory bulb, substantia nigra, blastocysts, embryo day 10.5, embryo day 6.5, embryo day 7.5, embryo day 8.5, embryo day 9.5, mammary gland, uterus, B220+B-cells, CD4+ Tcells, CD8+ Tcells, lymphnode, vomeronasal organ, thymus, kidney, retina	Catalyzes the NAD-dependent oxidative cleavage of spermidine
Insulin degrading enzyme (Ide)	NM_031156	19	Adipose tissue, adrenal gland, main olfactory, embryo day 6.5, embryo day 7.5, embryo day 9.5, fertilized egg, ovary, prostate, uterus, oocyte, heart, CD8+ Tcells, skeletal muscle, vomeronasal organ, salivary gland, tongue, thymus, trachea, bladder	Can cleave insulin and tgf-alpha
Interleukin 12a (IL12a)	NM_008351	3	Brown fat, bone, bonemarrow, preoptic, cerebellum, cerebral cortex, dorsal striatum, olfactory bulb, spinal cord upper, substantia nigra, embryo day 10.5, embryo day 6.5, embryo day 7.5, embryo day 8.5, embryo day 9.5, fertilized egg, B220+B-cells, CD8+ Tcells, lymphnode, skeletal muscle, spleen, thyroid	Cytokine that can act as a growth factor for activated T and NK cells (by similarity)
Helicase, mus308-like (Drosophila) (Hel308)	BC082601	5E	Fertilized egg, oocyte	ATP binding, DNA metabolism, helicase activity, hydrolase activity, RNA binding, single-stranded DNA-dependent ATP-dependent DNA helicase activity
Nuclear receptor subfamily 3, group C, member 1 (Nr3c1)	NM_008173	18	Brown fat, Adipose tissue, frontal cortex, preoptic, cerebellum, main olfactory epithelium, ovary, placenta, umbilical cord, large intestine, B220+B-cells, CD4+ Tcells, CD8+ Tcells, lung, skeletal muscle, epidermis, thymus, trachea, kidney	Receptor for glucocorticoids (gc)

Determination of PGE in the thymus

Promiscuous gene expression is currently identified on the basis of data from microarray analysis for the different mouse organs using combined information from the public database GNF Gene Expression Atlas (http://symatlas.gnf.org/SymAtlas) (Su et al. 2004). This data bank presents gene expression in more than 60 mouse tissues/organs as assessed by gene array analysis using Affymetrics microarrays. Data information include GenBank accession, chromosomal location and molecular/biological function of each gene analysed (Table I).

At present, we are considering only the promiscuous genes whose expression was significantly induced in the thymus (as displayed by the SAM algorithm) and detected in different organs or tissues, besides the thymus, and whose expression levels were greater than median in relation to all other organs which appear in the GNF Atlas.

Emergence of PGE during thymus ontogeny

The complexity of PGE depends on increases in ascending order from cTECs, to immature mTECs, to mature $CD80^{hi}$ mTECs. These different gene pools are not complementary but additive, that is, there is no apparent association between the respective molecular/biological functions of the genes in parenchymal organs. The significance of PGE in the thymus is associated with central tolerance (Sospedra et al. 1998; Bruno et al. 2002, 2004) persisting during the entire exporting period of T cells from the thymus (Derbinski et al. 2001).

In fact, self-tolerance induction is dependent on developmentally regulated key processes, such as expression of TCR on the surface of lymphocytes allowing for TCR–MHC–peptide recognition, which enables the positive/negative selection of T cells in fetal thymus (Kyewski and Derbinski 2004).

Studies with freshly obtained fetal thymus allowed for the demarcation of the emergence of TCR beta V(D)J recombination during *in vivo* thymus ontogeny among different inbred mouse strains, contributing to revealing the effect of the genetic background on T cell development (Macedo et al. 1999; Espanhol et al. 2004; Cardoso et al. 2006).

The control of gene expression during the development of this organ has gained priority among several research teams, including our own group (Espanhol et al. 2004; Magalhães et al. 2005; Cardoso et al. 2006) with the objective of developing a clearer understanding the molecular mechanism of the central tolerance induction.

The cDNA microarray method has permitted the possibility of analysing thousands of genes at once and of performing a true dissection of this organ by means of transcript identification, characterizing virtually all the main cell types populating the thymus (Puthier et al. 2004).

In a recent study, using the cDNA microarray method, we identified the *in vivo* modulation of several cell-signaling genes, including those of the calcium cascade pathway, which is important for individual stages of T cell maturation and the control of anergy during murine thymus ontogeny (Puthier et al. 2004).

Using the same kind of analysis, our group recently showed the modulation of gene expression in murine fetal thymus organ cultures (FTOCs), at transcriptome scale and revealed an overlap between genes associated with TCR V(D)J recombination and DNA repair. We demonstrated that the association of FTOC cultures and cDNA microarray technology allows for sufficient accuracy to uncover the participation of essential genes implicated in thymus development (Cardoso et al. 2006)

Considering that the expression of TCR in the surface of maturing T cells is a key feature, allowing the recognition of MHC-peptide during positive/negative selection, we are currently employing cDNA microarrays to observe the extent of PGE when the TCR V beta gene (TRBV8.1) rearrangement emerges during the *in vivo* development of the thymus of Balb-c, C57Bl/6 and (Balb-c × c57Bl/6)F_1 mouse strains.

Comparing thymus gestation time as a result of fetal age, it was possible to determine the onset of gene induction, representing 57 different parenchymal and 7 lymphoid organs whose coded proteins are considered to be parenchymal organ antigens, thus indicating the occurrence of PGE.

Thymus transcriptome profiling was assessed using glass slide cDNA microarrays containing 4500 IMAGE thymus target sequences hybridized with fluorescent Cy3 or Cy5 cDNA probes. PGE was identified on the basis of gene expression data for the different parenchymal organs.

To identify significant changes in the gene expression of fetal thymus when TRBV8.1 V(D)J recombination occurred compared to a reference pool, we used a scatter plot of the observed relative difference $d(i)$ vs. the expected relative difference $d_E(i)$ as shown by the SAM program.

Most of the 4500 sequences tested presented $d(i) \cong d_E(i)$, indicating that their expression pattern remained unaltered. However, some genes were significantly repressed (71 in Balb-c, 57 in C57Bl/6 and 17 genes in (Balb-c × C57Bl/6)F_1) or induced (3 in C57Bl/6 and 70 genes in (Balb-c × C57Bl/6)F_1).

In this study, we defined only the induced genes as a manifestation of PGE (Table I). Moreover, 2 induced ESTs were found in C57Bl/6 and 38 in (Balb-c × C57Bl/6)F_1 but, due to their actual status of unknown function or organ representation, these sequences were not considered. This is evidence that the extent of PGE is greater than observed in this study.

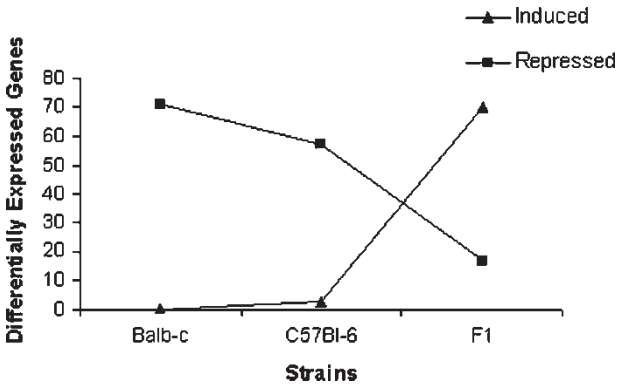

Figure 1. Number of significant induced or repressed genes in the fetal thymus during the emergence of TRBV8.1 recombination among inbred mouse strains. F1 = (Balb-c × C57Bl/6)F_1.

Interestingly, the microarray design and statistical stringency used in the SAM program allowed us to observe the manifestation of PGE at the onset of TRVB8.1 V(D)J recombination but this was observed only in the C57Bl/6 strain and the (Balb-c × C57Bl/6)F_1 hybrid. In the same developmental period, the Balb-c strain did not exhibit PGE, considering the statistical stringency we used in this study (Table I).

Figure 1 shows that among the strains studied, significant induction is inversely proportional to gene repression, strongly suggesting a role for the genetic background of strains in the control of PGE. Figure 2 illustrates that the reproductive and central nervous systems and stem cells/glands in C57Bl/6 and the central nervous and reproductive systems and stem cells in (Balb-c × C57Bl/6)F_1 are the predominant parenchymal organs most represented in the thymus in this phase of development, followed by the lymphoid system.

To our knowledge, these findings represent the first association study between the emergence of a TR gene V(D)J recombination (TRVB8.1) and occurrence of PGE among inbred mouse strains, with implications regarding the fine demarcation of key processes of self-tolerance induction.

Parenchymal organ representation in the thymus

The 3 induced genes in C57Bl/6 and 71 in (Balb-c × C57Bl/6)F_1 identified as significantly induced at 14–15 days p.c. were assigned to 57 parenchymal and 7 lymphoid organs according to their predominant expression, which were subgrouped in 17 systems.

Chromosomal location of the differentially expressed genes

The genomic distribution of the significantly modulated genes (repressed and induced), 71 in Balb-c, 60 in C57Bl/6 and 87 in (Balb-c × C57Bl/6)F_1, allowed for the organization of chromosomal clusters of coordinated expression.

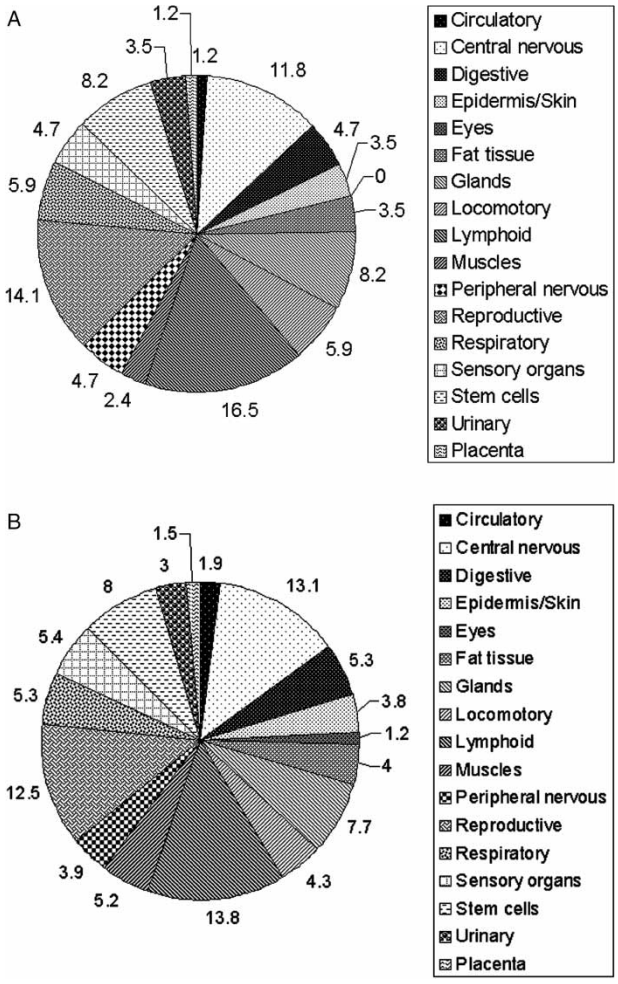

Figure 2. Representation of tissue/organ systems specific gene expression in the fetal thymus during the emergence of TRBV8.1 recombination. Analysis of 4500 sequences was performed using glass slide cDNA microarrays, whose significant induced genes were annotated characterizing the promiscuous expression, which allow self-representation of tissue specific antigens in the thymus. 2A = C57Bl/6, 2B = (Balb-c × C57Bl/6)F_1.

Figure 3 shows the frequency distribution of the repressed and induced genes among chromosomes. All chromosomes, except Y, harbor differentially expressed genes, with slightly biased distribution on chromosomes 2, 5, 11, 13, 17 and 19 for the repressed genes in Balb-c, on chromosomes 2, 3, 6, 9 and 11 for the repressed and 11, 13 and 17 for the induced in C57Bl/6, on chromosomes 2, 7, 9, 13 and 15 for the repressed and on chromosomes 2, 4, 5, 7, 10, 11, 18 and 19 for the induced genes in the (Balb-c × C57Bl/6)F_1 hybrid.

Discussion and perspective

Self-tolerance induction should occur early, during the fetal development of the thymus, preventing

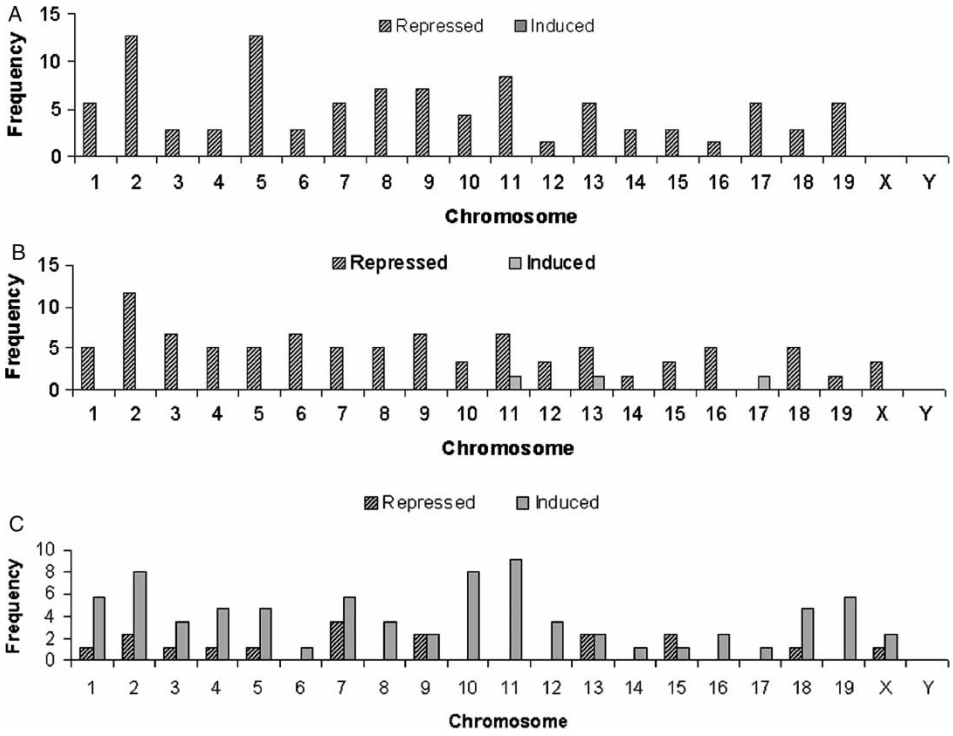

Figure 3. Chromosomal distribution of the repressed and induced genes in the fetal thymus during the emergence of TRBV8.1 recombination among inbred mouse strains. 3A = Balb-c, 3B = C57Bl/6, 3C = (Balb-c × C57Bl/6)F_1.

autoimmune pathological reactions. This phenomenon is dependent on the presentation of self-antigens to maturing T cells through TCR-MHC-peptide recognition (Sprent and Webb 1995; Hanahan 1998; Kishimoto and Sprent 2000). The assembling of functional TR genes, allowing for the expression of TCR on the surface of T cells, is dependent on the temporal emergence of TR gene V(D)J recombination, during the fetal development of the thymus (Junta and Passos 1998; Macedo et al. 1999; Espanhol et al. 2004).

The extent of self-representation of most parenchymal tissues and organs is guaranteed by PGE in the thymus, a phenomenon that is exhibited by mTECs, is complex and involves 5–10% of all known genes in mice and humans. Accordingly, the complexity of PGE increases in ascending order, from cTECs to immature mTECs to mature $CD80^{hi}$ mTECs. These different gene pools are not complementary but additive, that is, there is no apparent association between the respective molecular/biological function of the genes in parenchymal organs. The significance of PGE in the thymus is associated with central tolerance of T cells (Sospedra et al. 1998; Bruno et al. 2002; Kyewski and Derbinski 2004). While PGE by mTECs was well characterized by the authors cited above, its time course during thymus ontogenetic development requires further exploration.

Molecular characterization of PGE during fetal development of different inbred mouse strains is a relevant approach in immunobiology, since this system can represent a potential tool in clarifying this question and in evaluating whether the different genetic backgrounds play a role in the control of PGE.

Moreover, knockout (KO) mice could reveal evidence for the role of specific genes in this process.

Evidence obtained by our team shows, for the first time, the occurrence of PGE *in vivo* during the fetal development of the thymus. To evaluate whether PGE in this model system is a developmental dependent phenomenon, we regarded the differential gene expression during ontogeny through the comparison of gestation days (p.c.), as the most informative in the delineation of the gene pool.

Moreover, we compared two different inbred mouse strains expressing different MHC haplotypes, Balb-c = H-2d and C57Bl/6 = H-2K and their hybrid (Balb-c × C57Bl/6)F_1 demonstrating that PGE emerges on different days among the strains studied. These findings strongly suggest a role for the genetic background of these strains in the control of the emergence and extent of PGE.

Our results show that PGE occurs during thymus maturation; after TRBV8.1 gene V(D)J recombination in Balb-c and coinciding with TRBV8.1 V(D)J recombination in C57Bl/6 and in (Balb-c × C57Bl/6)F_1.

The early fetal thymus, by 13–15 days p.c., is mainly composed of homogeneous double-negative (DN) $CD4^-CD8^-$T cell precursors. By day 18 p.c. this population gradually acquires the CD4 marker

resembling the adult $CD4^{low}$ precursor (Shortman and Wu 1996).

These features allow for TCR-MHC-peptide recognition and enable the positive/negative selection of T cells in fetal thymus.

Our evidence for the occurrence of PGE in the late fetal thymus can be associated with the timing of the molecular events of T cell tolerance induction during ontogeny.

The data collected here were obtained by the cDNA microarray method, with the expression of the 4500 mouse mRNA sequences analyzed by the SAM algorithm (20). We found statistically significant gene modulation showing 71 repressed genes in Balb-c, 57 repressed and 3 induced genes in C57Bl/6 and 17 repressed and 70 induced genes in (Balb-c × C57Bl/6)F_1. Moreover, the significantly induced genes were indicative of emergence of PGE (Table I).

While the experiments were not conducted with cell-sorted purified mTECs, this caused no problems in the detection of differential TSA gene expression in the thymus. In order to bypass this potential difficulty, we used a cDNA microarray method, including a dedicated statistical algorithm for data analysis (SAM algorithm), which presented sufficient accuracy to distinguish and quantify TSA gene expression originating from thymic epithelial cells, especially mTECs (Gotter et al. 2004, Kyewski and Derbinski 2004; Derbinski et al. 2005).

In fact, cDNA microarray data mining has permitted the virtual dissection of the mouse thymus into its principal cellular components by means of the identification of the specific cellular transcripts (mRNAs) (Puthier et al. 2004). These observations demonstrate the feasibility of the use of whole thymus as starting material in PGE studies.

In agreement with previous observations (Derbinski et al. 1995) the molecular/biological function of promiscuously expressed genes found in our model system, showed no interrelationship (Table I).

Regarding the chromosomal localization of the repressed and induced genes, no important preferential distribution was identified. All chromosomes harbor promiscuously expressed genes with slightly biased distribution on chromosomes 2, 5, 10 and 11 among the repressed genes and on chromosomes 2, 10 and 11 among the induced genes. The exception was chromosome Y on which no repressed or induced gene was positioned considering the statistical stringency used in this study. (Figure 3).

This feature of random PGE distribution in the genome, strongly suggests an uncommon model of gene regulation found in the thymus that needs further study.

In this regard, certain urgent questions remain, like whether the chromatin scaffold in mTECs is so arranged to allow for promiscuous gene expression and whether the methylation pattern of mouse genome controls this phenomenon.

Finally, the use of the fetal thymus model system and cDNA microarray method, open up perspectives to determine the modulation and extent of PGE in autoimmune diseases, through the analysis of genetically compromised mouse strains.

During preparing this article, the Science magazine (Sciencexpress Report) published online the evidence for a functional second thymus in mice, located in the neck, whose observations were done and communicated by Dr Rodewald's group of the University of Ulm, Ulm, Germany (Terszowski et al. 2006). Once broadly recognized the existence of the second thymus, this observation also provide perspective for reevaluating the physiological relevance of extra-thymic T cell development, as stated the authors and evaluate the occurrence of PGE in this organ.

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