MINI-REVIEW

Quantification of T cell receptor rearrangement excision circles to estimate thymic function: an important new tool for endocrine–immune physiology

V Geenen, J-F Poulin1,2, M L Dion1,3, H Martens, E Castermans, I Hansenne, M Moutschen, R P Sékaly1,4 and R Cheynier1

University of Liege Centre of Immunology, Institute of Pathology CHU-B23, Department of Medicine, Division of Endocrinology, CHU-B35, B-4000 Liege-Sart Tilman, Belgium
1Laboratoire d’Immunologie, Centre de Recherches du CHUM, Hôtel-Dieu, Montreal, Quebec, Canada
2Department of Medicine, Division of Experimental Medicine, McGill University, Montreal, Quebec, Canada
3Department of Microbiology and Immunology, McGill University, Montreal, Quebec, Canada
4Département de Microbiologie et Immunologie, Faculté de Médecine, Université de Montréal, Québec, Canada
(Requests for offprints should be addressed to V Geenen; Email: vgeenen@ulg.ac.be)
(V Geenen and J-F Poulin contributed equally to this work)
(R Cheynier is now at Laboratoire d’Immunologie Cellulaire Antivirale, Institut Pasteur, 28 rue du Dr Roux, F-75015, Paris, France)

Abstract

Although the thymus constitutes a target organ for most protein and steroid hormones, it has been quite difficult to determine the precise control exerted in vivo by the endocrine system upon thymic function. The biological role of the thymus is to ensure the generation of a diversified population of peripheral T cells able to respond to non-self-antigens but nevertheless tolerant to self-antigens. For a long time, thymic function could not be monitored, as a consequence of the absence of adequate technology to differentiate recent thymic emigrants from naive T cells. The generation of T cell receptor (TCR) diversity occurs in the thymus through recombination of gene segments encoding the variable parts of the TCR α and β chains. During these processes, by-products of the rearrangements are generated in the form of TCR excision circles (TRECs). As these molecules are lost upon further cell division, their quantification is actually considered as a very valuable tool to estimate thymic function. The most appropriate TREC is δRec-ΨJa TREC or signal joint TREC resulting from δRec-ΨJa rearrangement (TCRD deletion) that occurs late during thymopoiesis, before Vα-Jα rearrangement. Here we describe how TREC quantification is a powerful and reliable method to evaluate the impact of hormones and endocrine disorders upon thymic function.


The place of the thymus in the endocrine system

During embryonic development, the thymic and inferior parathyroid epithelial rudiments both originate from the endoderm of the third pharyngeal pouch. The thymic epithelial anlage is then colonised by cells of haematopoietic origin (thymocytes) and dendritic cells. Independently, it is invaded by macrophages, also of bone marrow origin. The thymus was considered for a long time to be a component of the endocrine system, but the model of endocrine signalling failed to accurately describe the interactions between the thymic stromal network and developing T lymphocytes (thymocytes). The involution of the thymus following hypophysectomy was the first evidence for the role of an important endocrine gland in the control of the immune system (Smith 1930). Aside from their endocrine and metabolic functions, antehypophysial and neurohypophysial hormones exert trophic actions on thymus and T cell development (extensively reviewed in Besedowsky & Del Rey 1996, Savino & Dardenne 2000, Berczi 2001). Despite strong evidence indicating regulation of haematopoiesis by growth hormone (GH) (Blazar et al. 1995, Golde & Cline 1977, Golde et al. 1977, Tian et al. 1998), it remains to be found whether these GH effects are mediated through either modulation of proliferation, activation or survival of
haematopoietic cells. Interestingly, GH administration increases thymic volume and density in HIV-infected patients (Napolitano et al. 2002). Moreover, GH-induced increase in thymic mass was associated with an increase in naïve T cells. Combined with the fact that discontinuation of GH therapy led to the recurrence of thymic atrophy, this strongly suggests that thymopoiesis is enhanced by GH treatment (Napolitano et al. 2002). Although GH receptor is detected on a variety of bone marrow–derived cells, most of the effects of GH on the thymus result from a paracrine action of GH–dependent insulin–like growth factor-I (Kecha et al. 2000).

Involution of the thymus is observed in experimentally induced hypothyroidism (Fabris 1973, Abou-Rabia & Kendall 1994). Thymic hyperplasia and intrathymic expression of thyrotrophin receptor have been observed in patients with Graves’ disease (Paschke & Geenen 1995, Murakami et al. 1996).

The impact of steroid hormones on thymic function has also been richly documented. Receptors for glucocorticoids, gonadal steroids and vitamin D are expressed on both thymic epithelial cells and thymocytes. Fluctuation in androgen levels leads to striking changes in thymus weight, cellularity and cell composition. The thymus of male mice is enlarged after orchidectomy or under conditions of defective androgen action (Greenstein et al. 1987). Androgen receptors are expressed by thymic epithelial cells and mediate androgen action on T cell development (reviewed in Olsen & Kovacs 1996, Olsen et al. 2001). By 1936, Selye had already observed the marked involution of the thymus and adrenal hypertrophy after severe injury (Selye 1936). Beyond the well–established pro-apoptotic effects of glucocorticoids on thymocytes (Cidlowski et al. 1996), the tight control of immune function and thymopoiesis by the corticotroph axis has been evidenced in physiological conditions and inflammatory disorders (reviewed in Chrousos 2000, Webster et al. 2002).

Physiology of the thymus

The prominent role of the thymus in T cell generation (thymopoiesis) was demonstrated by pioneering studies conducted by Miller in the early 1960s (Miller 1961). The critical role of the thymus in the establishment of central T cell self–tolerance by clonal deletion (negative selection) was evidenced more recently (Kappler et al. 1987, Kisielow et al. 1988, Nossal 1994). The integration of the thymus in endocrine physiology did not completely vanish, however, and the primary lymphoid organ is crucially located at the crossroad between the major systems of cell–to–cell communication, the neuroendocrine and immune systems. Through transcription of neuroendocrine genes in the thymus stromal network and expression of cognate receptors by immature T cells, the neuroendocrine system regulates early T cell differenti-
antigen likely to be encountered, the peripheral T cell population must be highly diverse. This huge heterogeneity, which is not encoded in the germ-line, is generated during T cell differentiation in the thymus. In this organ, T cell progenitors undergo a series of developmental events that can be monitored by membrane expression of cluster differentiation (CD) antigens. During αβ (and γδ) T cell maturation, the juxtaposition of various gene segments leads to the generation of TCRA and TCRB chains (TCRG and TCRD). The antigen-recognition variable domains of TCR α and β chains are encoded by combinations of variable (V), diversity (D), and joining (J) gene segments (TCR β chains), or V and J gene segments (TCR α chains). V(D)J recombination is initiated by the recognition of recombination signal sequences (RSSs) that flank the coding segments. RSSs consist of conserved heptamer (CACAGTG) and nonamer (ACAAAAACC) sequences separated by a less conserved spacer of 12 or 23 bp. During maturation, pre-T cells transiently express the RAG1 and RAG2 genes in order to fuse together V, D, and J segments. Two successive waves of RAG1 and RAG2 expression coincide with TCRB and TCRA chain rearrangements. RAG-1 and RAG-2 cooperate to recognize the RSSs and introduce hair-pinned site-specific cleavages at the signal–coding borders of a given pair of coding segments. Fusion between distinct V, D, and J segments leads to a vast array of differently diversified TCR β chain nucleotide sequences. While this contributes in generating considerable TCR diversity, during any rearrangement process, the DNA located between the two RSSs is excised through other recombination events that will not generate an sjTREC molecule (Verschuren et al. 1997). Therefore, peripheral blood quantification of sjTREC frequencies leads to an underestimate of the real frequency of RTEs. Nonetheless, it provides an unequivocal way to estimate the blood concentration of RTEs and thus, evaluate the TREC level in the periphery reflects RTE numbers and is largely accepted as a surrogate marker for thymic function (Kong et al. 1999).

In a recent paper, Hazenberg et al. (2000) suggested that TREC concentration should be utilised carefully to estimate thymic function as it can be affected by events occurring in the periphery such as T cell proliferation. So, data of TREC quantification should be cautiously interpreted in particular conditions such as during immune reconstitution following bone marrow transplantation or during HIV infection. However, in healthy individuals, only homeostatic naive T cell proliferation is likely to significantly affect peripheral T cell TREC content, as antigen-induced T cell proliferation only affects memory T cells and thus does not affect the RTE population. Different techniques are employed to measure TREC concentrations, such as real-time PCR, quantitative competitive PCR (Douek et al. 1998), and PCR-ELISA (Al-Harthi et al. 2000). Also, measurement units of TREC concentration vary, including TRECs/10⁶ peripheral blood mononuclear cells, TRECs per CD45RA+ T cell, TRECs/µg DNA of T cells, and TRECs/10⁵ CD4⁺ T cells.

Age-related evolution of sjTREC

Since the major part of thymocyte proliferation and expansion occurs before this TREC is formed, the sjTREC or δRec-Ψα TREC is a very appropriate choice to reflect thymopoiesis (Douek et al. 1998). Therefore, quantification of the sjTREC frequency was selected in our studies. A two-step multiplex real-time PCR was used to quantify sjTRECs in human individuals. In order to carefully quantify the sjTREC frequency, a multiplex nested real-time quantitative PCR was performed on blood samples. In the first step, both the sjTREC molecule and a fragment of the CD3γ chain were amplified, allowing cell quantification in the sample as described by J-F Poulin, M Sylvestre, P Champagne, M-L Dion, N Kettaf, A Dumont, M Lainesse, P Fontaine, D-C Roy, C Perreault, R-P Sékaly & R Cheynier (unpublished observations). In order to stay in the logarithmic linear phase of
the PCR for both amplicons, the first step of amplification was only performed for 22 cycles. The second part of the nested amplification was performed using LightCycler technology (Roche), allowing the quantification of both the sjTREC and the number of cells (as half of the CD3 \( \gamma \)/afii9828 copy number) initially present in each sample. These quantifications were based on the amplification of a plasmid reference standard containing both sjTREC and CD3 \( \gamma \)/afii9828 templates. For each sample, a precise number of sjTREC copies/100 000 cells can be deduced.

Through this methodology, sjTREC concentration was determined in 41 subjects ranging from 1 to 83 years old. As shown in Fig. 2, sjTREC concentration exponentially decreases with a half-life of 19·62 years. In the population sample, no significant difference appears in the number of sjTRECs/100 000 cells between the 1–10 year (483 ± 108 S.E.M., \( n=8 \)) and the 11–40 year groups (354 ± 77, \( n=16 \)) (Fig. 3). However, a very significant difference (\( P<0.001 \)) exists between the 10–40 year and the 41–60 year groups (65 ± 27, \( n=10 \)). This decrease remains significant (\( P<0.001 \)) between the 41–60 year and the 61–80+ year (20 ± 5, \( n=7 \)) groups. In our hands, a correlation with gender was not found although such a correlation was previously reported (Pido-Lopez et al. 2001).

**Thymic function in clinical conditions and in the elderly**

In T cell depleting diseases such as HIV infection, treatment with highly active anti-retroviral therapy (HAART) induces a rapid increase in the numbers of detectable TREC in peripheral cells (Douek et al. 1998, Poulin & Sekaly 1999, Poulin et al. 1999). Thus, the adult thymus retains a significant thymopoietic function and still generates naive, newly differentiated, and functional T cells for export to the periphery (Jamieson et al. 1999). Increases in naive T cells in HIV-infected and HAART-treated patients who had been thymectomised before suggest that naive T cells may replicate in the periphery (Haynes et al. 1999). Also in immuno-haematologic diseases and...
malignancies, rapid T cell regeneration after high-dose chemotherapy requires a residual thymic function (Mackall et al. 1995). An efficient thymic function was shown to be associated with a favourable immune reconstitution after cord blood stem cell transplantation (Talvensaari et al. 2002). In patients with severe combined immunodeficiency, stem-cell transplantation (Patel et al. 2000) or gene therapy (Hacein-Bey-Abina et al. 2002) was followed by an increase from undetected levels in the number of T cells containing sjTRECs. Some thymic dysfunction in T cell generation was also recently suggested in rheumatoid arthritis (Koetz et al. 2000).

For a long time it has been assumed that the thymic function gradually decreases after puberty, in parallel with the progressive enrichment of the thymic stroma in adipose cells (thymic adipose ‘involution’). By 18 years of age, the periphery was thought to be seeded with a complete repertoire of antigen-reactive T cells (Simpson et al. 1975). In recent years, more and more data have contradicted this quite dogmatic view and provided demonstrative evidence that the adult thymus remains active late in life and generates functional T cells for the peripheral lymphoid repertoire (Aspinall et al. 2002). In accord with those studies, as shown in Fig. 3, a significant number of sjTRECs are measured even in adults between 61 and more than 80 years of age. The persistence of thymopoiesis and generation of TCR diversity has important implications for the problem of immunosenescence. Indeed, ageing of the immune system is associated with increased morbidity and mortality from infectious diseases, decreased tumoral immunity with higher prevalence of cancers, as well as a decrease of immune self-tolerance leading to some autoimmune diseases (Pawelec et al. 2002). The restoration of thymic function in ageing and in various disorders seems thus to be an important objective in the elderly, in AIDS and in a series of haematological diseases. Since the thymic stroma expresses receptors for a number of hormones (reviewed in Kelley et al. 1986; Savino & Dardenne 2000, Murphy & Longo 2000), ensuring an adequate endocrine equilibrium should be important for maintaining thymic functions, i.e. generation of T cells with a diverse but self-tolerant TCR repertoire. Obviously, quantification of thymic T cell generation through TREC methodology now constitutes a very important parameter to measure in studies aiming to evaluate the consequence of neuroendocrine ageing (Lamberts et al. 1997) and diseases upon immune function, as well as the benefits expected to follow restoration of hormonal equilibrium.

Acknowledgements

These studies are supported by the Research Special Fund of Liege University, by Fondation Léon Fredericq, by the Fund for Scientific Medical Research of Belgium (FRSM grant no. 3.4574.02), by Fondation Vaugrenier for Tolerance Research, and by the Belgian Federation against Cancer. V G is Research Director of the Belgian NFSR; I H is supported by FRIA; J-F P is a recipient of a CHIR doctoral research award.

References


Ashwell JD, Lu FWM & Vecchio MS 2000 Glucocorticoids in T cell
development and function. Annual Review of Immunology 18
304–348.
Aspillar R, Andrew D & Pido-Lopez J 2002 Age–associated changes
Frontier of Biology Series, vol 1, pp 3–45. Eds I Berczi & RM
Besedowski HO & Del Rey A 1996 Immune-neuro-endocrine
Blazar BR, Brennan CA, Broxmeyer HE, Shultz LD & Valler DA
1995 Transgenic mice expressing either bovine growth hormone
(bGH) or human GH releasing hormone (hGRH) have increased
splenic progenitor cell colony formation and DNA synthesis in vitro
and in vivo. Experimental Hematology 23 1397–1406.
Chrousos GP 2000 The stress response and immune function; clinical
implications. The 1999 Novera H Spector Lecture. Annals of the
Cidlowski JA, King KL, Evans-Storms RB, Montague JW, Bortner
CD & Hughes FM Jr 1996 The biochemistry and molecular
biology of glucocorticoid-induced apoptosis in the immune system.
Recent Progress in Hormone Research 51 457–490.
De Villartay JP, Hockett RD, Coran D, Korsmeyer SJ & Cohen DI
1988 Deletion of the human T-cell receptor β gene by a
Douek DC, McFarland RD, Keiser PH, Gage EA, Massey JM,
1998 Changes in thymic function with age and during treatment of
Fabris N 1973 Immunodepression in thyroid-deprived animals. Clinical
and Experimental Immunology 15 601–611.
Fugmann SD, Lee AF, Schlochter PE, Villey IJ & Schatz DG 2000
The RAG proteins and V(D)J recombination: complexes, ends and
transposition. Annual Review of Immunology 18 495–522.
Geenen V, Kecha O, Briot F, Charlet-Renard C & Martens H 1999
The thymic repertoire of neuroendocrine self-antigens: biological
role in T cell selection and pharmacological implications.
Neuroimmunomodulation 6 115–125.
Geenen V, Briot F, Hansenne I, Kecha-Kamoun O & Martens H
Gold DE & Cline MJ 1977 Hormonal interactions with
Gold DE, Bersch N & Li CH 1977 Growth hormone:
species-specific stimulation of erythropoiesis in vitro. Science 196
1112–1113.
Greenstein BD, Fitzpatrick FT, Kendall MD & Wheeler MJ 1987
Regeneration of the thymus in old mice treated with a stable
Hacein-Bey-Abina S, Le Deist F, Carlier F, Bouneaud C, Hue C, De
S et al. 2002 Sustained correction of X-linked severe combined
immunodeficiency by ex vivo gene therapy. New England Journal of
Medicine 346 1185–1193.
Haynes BF, Hale LP, Weinhold KJ, Patel DD, Liao HX, Bressler PB,
1999 Analysis of the adult thymus in reconstitution of T
lymphocytes in HIV-1 infection. Journal of Clinical Investigation 103
453–460.
Hazenberg MD, Otto SA, Cohen Stuart JW, Verschuren MC,
Borleffs JC, Boucher CA, Coutinho RA, Lange JM, Rinke de Wit
TF, Tsegey A et al. 2000 Increased cell division but not thymic
dysfunction rapidly affects the T-cell receptor excision circle
content of the naive T cell population in HIV-1 infection. Nature
Medicine 6 1036–1042.
Hedden B & Mason D 2000 The third function of the thymus.
Immunology Today 21 95–99.
Huang J, Durum SK & Muegge K 2001 Cutting edge: histone
acetylation and recombination at the TCR gamma locus follows
IL-7 induction. Journal of Immunology 167 6075–6077.
Jameson BD, Douek DC, Killian S, Hultin LE, Scripture-Adams DD,
Giorgi JV, Marelli B, Koup RA & Zack JA 1999 Generation of
Kappler JW, Roehm N & Marrack P 1987 T-cell tolerance by clonal
Kecha O, Briot F, Martens H, Franchimont N, Renard C, Greumers
R, Defresne MP, Winkler R & Geenen V 2000 Involvement of
insulin-like growth factors in early T cell development: a study
using fetal thymic organ cultures. Endocrinology 141 1209–1217.
Walker EB 1986 GH: pituitary adenoma cells can reverse thymic
aging in rats. PNAS 83 5663–5667.
Kisielow P, Blüthmann H, Stærzer D, Steinmetz M & Von Boehen H
1988 Tolerance in T-cell receptor transgenic mice involves deletion
Koetz K, Brey E, Spiekschen K, O’Fallon WM, Gorozy J & Weyand
CM 2000 T cell homeostasis in patients with rheumatoid arthritis.
PNAS 97 9203–9208.
Kong FK, Chen CL, Six A, Hockett RD & Cooper MD 1999 T-cell
receptor deletion circles identify recent thymic emigrants in the
peripheral T cell pool. PNAS 96 1536–1540.
Lamberts SWJ, van den Beld AW & van der Lely AJ 1997 The
Mackall CL, Fleischer TA, Brown MR, Andrich MP, Chen CC,
Feuerstein IM, Horowitz ME, Magrath IT, Shad AT, Steinberg SM
et al. 1995 Age, thymopoiesis, and CD4+ T-lymphocyte
regeneration after intensive chemotherapy. New England Journal of
Medicine 332 143–149.
Martens H, Magrange B, Robert F, Charlet C, De Groote D,
Heymann D, Godard A, Soulliou JP, Moonen G & Geenen V
1996a Cytokine production by human thymic epithelial cells:
control by the immune recognition of the neurohypophysial
Martens H, Goxe B & Geenen V 1996b The thymic repertoire of
neuroendocrine-related self-antigens: physiological implications in
T-cell life and death. Immunology Today 17 312–317.
Miller JFAP 1961 Immunological function of the thymus. Lancet 2
748–749.
Murakami M, Hosoi Y, Negishi T, Kaniya Y, Yamada M,
Iriuchijima T, Yokoo H, Yoshida Y & Mori M 1996 Thymic
hyperplasia in patients with Graves’ disease. Identification of
dihydropyrimidine receptors in human thymus. Journal of Clinical
Investigation 98 2228–2234.
Murphy WJ & Longo DL 2000 Growth hormone as an
immunomodulating therapeutic agent. Immunology Today 21
211–213.
Napolitano LA, Lo JC, Gotway MB, Mulligan K, Barbour JD,
Schmidt D, Grant RM, Halvorsen RA, Schambelan M & McCune
JM 2002 Increased thymic mass and circulating naive CD4 T cells
in HIV-1-infected adults treated with growth hormone. AIDS
16 1103–1111.
Nossal GJV 1994 Negative selection of thymocytes. Cell 76
229–239.
Olsen NJ & Kovacs WJ 1996 Gonadal steroids and immunity.
Endocrine Reviews 17 363–384.
Olsen NJ, Olson G, Viselli SM, Gu X & Kovacs WJ 2001 Androgen
receptors in thymic epithelium modulate thymus size and
Paschke R & Geenen V 1995 Messenger RNA expression for a TSH
receptor variant in the thymus of a two-year-old child. Journal of
Molecular Medicine 73 577–580.
Patel DD, Gooding ME, Parrott RE, Curtis KM, Haynes BF &
Buckley RH 2000 Thymic function after hematopoietic stem-cell
transplantation for the treatment of severe combined


Smith PE 1930 Effects of hypophysectomy upon the involution of the thymus in the rat. *Anatomical Recordings* **47** 119–129.


Received 24 September 2002
Accepted 4 December 2002