Thymic Function in Juvenile Idiopathic Arthritis

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Abstract

Objective Thymic function declines exponentially with age. Impaired thymic function has been associated with autoimmune disease in adults but has never been formally assessed in childhood autoimmunity. We therefore determined thymic function in children with the autoimmune disease Juvenile Idiopathic Arthritis (JIA).

Methods We measured thymic function in 70 children and young adults with JIA (age range 2.1 – 30.8 (median 10.4)) and 110 healthy age matched controls using four independent assays. We quantified T-cell receptor excision circles (WBLogTREC/ml), the proportion of $CD4^+$ $CD45RA^+CD31^+$ T-cells (representing recent thymic emigrants - %RTE) and measured intra-thymic proliferation by calculating the $\alpha TREC/\Sigma\beta TREC$ ratio. Lastly, regulatory T-cells (T_{Reg}) of thymic origin ($CD4^+FOXP3^+$) were quantified in peripheral blood to assess the ability of the thymus in JIA to generate this T-cell subset.

Results Thymic function was equivalent by all four parameters in JIA when compared with our control population. Furthermore, there was no consistent effect of JIA subtype on thymic function, although intra-thymic proliferation was higher in the small RF^+polyarticular group. There were no significant effects of disease modifying anti-rheumatic drugs (DMARDs) or oral corticosteroids on thymic function, although those with the worst prognostic ILAR subtypes were also those most likely to be on a DMARD.

Conclusions We demonstrate that children and young adults with JIA, unlike adults with autoimmune diseases, have thymic function that is comparable with healthy controls. The varied pathologies represented by the term 'JIA' suggest this observation may not be disease specific and raises interesting questions about the aetiology of thymic impairment in adult autoimmunity.

Introduction

The thymus plays a critical role in the development of normal immune tolerance (1). As well as negatively selecting potentially autoreactive T-cells, it also positively selects the naturally occurring regulatory T-cell subset (T_{Reg}) (CD4$^+$FOXP3$^+$) (2). Cross-sectional studies have demonstrated impaired thymic function in several adult autoimmune diseases (3-6). It remains unclear whether the thymic defect is primary or secondary, although single gene defects affecting thymic integrity can predispose to autoimmunity (7, 8). In contrast to adults, thymic function has not been formally quantified in childhood autoimmunity such as JIA.

The normal thymus is largest in childhood and is well known to decline in size and function with increasing age (9). Whilst not normally of apparent consequence, this decline becomes important under circumstances where the T-cell pool is depleted, such as in HIV-AIDS or during lympho-ablative treatments such as bone marrow transplant (BMT) or stem cell transplantation (SCT). This [normal] decline in thymic function with aging is thought to explain the slower and often less complete T-cell reconstitution following SCT in adults when compared with that in children (10, 11).

JIA describes a heterogeneous group of diseases (12) ranging in severity from severe polyarticular disease sometimes associated with systemic features, to more benign oligoarticular disease. The more severe JIA subtypes have a poor prognosis with lack of response to intense immunosuppression in some cases (13, 14). Autologous SCT has recently been shown to induce prolonged, drug free remission in a proportion of such refractory patients (15).

Measuring thymic function in humans is complicated by the lack of a specific phenotype for recent thymic emigrants (RTE), although the surface profile CD4$^+$CD45RA$^+$CD31$^+$ has been proposed as a potential marker (16). Rearrangement

of the T-cell receptor alpha chain gene results in the formation of an episome of DNA called a 'T cell receptor excision circle' (TREC) (17). TREC are present only in thymically-derived T-cells and do not divide with cellular mitosis. When quantified per millilitre of whole blood, TREC are widely accepted to be the optimal measurement of thymic function (18).

In the current study we compare thymic function in children with JIA and healthy controls. By measuring TRECs, the proportion of $CD4^+CD45RA^+CD31^+$ T-cells and T_{Reg} in peripheral blood, and intra-thymic T-cell development using a novel assay, we demonstrate that thymic function is not compromised in JIA.

Patients and Methods

Study subjects

Following ethical approval and informed consent from the child and/or a parent or guardian, 9ml blood samples were obtained from healthy control children undergoing simple surgical procedures. Samples from young adults were donated by healthy volunteers. Study subjects were consecutive attendees at the Newcastle Regional Paediatric Rheumatology Service in Newcastle. All were given a diagnosis of JIA classified according to the ILAR classification by a consultant Paediatric Rheumatologist (12). Samples from older patients were obtained at adolescent and young adult rheumatology clinics. Blood was collected into Vacuette EDTA K3 tubes (Greiner Bio-one, Austria).

DNA extraction

DNA was extracted from 300μl whole blood using the Wizard Genomic DNA extraction kit ® (Promega) and stored at 4°C. Sample purity and quantity was determined by spectrophotometry (Nanodrop® ND-100).

TREC Quantification

We have developed a method quantifying TREC/ml in DNA extracted from 300μl whole blood (manuscript submitted). Briefly, WBLogTREC/ml is determined from a simultaneously amplified standard curve (range 10^7- 10^1 TREC molecules) using quantitative real time PCR (RQ-PCR), ABI Prism 7900HT Sequence Detector System and SDS2.2 software (Applied Biosystems, Warrington, UK).

25μl reactions contained primers CACATCCCTTTCAACCATGCT and GCCAGCTGCAGGGTTTAGG both at 700nM, 150nM Taqman hydrolysis probe (6-FAM-ACACCTCTGGTTTTTGTAAAGGTGCCCACT-TAMRA), 12.5μl JumpStart™ Taq ReadyMix (SIGMA) and 200ng DNA. Thermal cycling conditions were 50°C for 2 minutes then 95°C for 10 minutes, then 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. Experimental samples were run in duplicate and averaged.

Quantification of intra-thymic proliferation

Intra-thymic proliferation was measured using a modified version (to allow for use with SYBR Green) of the method published by Dion *et al* (19). Ten TCRβ chain $D_\beta \rightarrow J_\beta$ rearrangements (βTREC) and the TCRα chain δrec→ψJα rearrangement (αTREC) were quantified in separate, nested PCRs. A duplex first round reaction with

primers for individual βTREC and genomic CD3 was followed by individual RQ-PCRs. The CD3 copy number multiplied by 0.5 (since each cell contains two copies) allowed βTREC frequency to be reported per 10^5 cells.

First round 25μl PCR reactions contained 1x High Fidelity PCR buffer (Invitrogen), 0.2mM each of dNTPs (Invitrogen), 1.5mM $MgCl_2$ (Invitrogen), 1U Platinum Taq High Fidelity (Invitrogen), 500nM of both primers (Sigma Genosys) and 150ng target DNA. Initial denaturation was at 95°C for ten minutes followed by 22 cycles of 95°C for 30s and 72°C for 30seconds. Second round, 25μl reactions contained 2X SybrGreen® PCR Master Mix (TAKARA), 400nM of each primer (SIGMA Genosys) and 10μl template. Thermal cycling conditions were 95°C for 10 seconds followed by 40 cycles of 95°C for 5 seconds and 60°C for 60 seconds.

Surface staining of PBMC for quantification of recent thymic emigrants

70μl of whole blood was incubated in the dark at room temperature for 15 minutes, with combinations of antibodies to surface antigens: CD31-PE, CD45RA-FITC (Serotec, Oxford UK) and CD4-PE-Cy5 (Beckton Dickinson). Stained samples were incubated with 1X FACS Lyse Solution ® (Beckton Dickinson) for 10 minutes and washed twice.

Isolation of PBMC from whole blood and intracellular cell staining

PBMC were isolated according to a standard, sucrose density gradient (Lymphoprep®) protocol and cryopreserved in 10% DMSO in FCS at -80°C until use. Thawed cells were surface stained with CD4+-FITC and then FOXP3-APC antibody (eBiosciences,Wembly, UK) according to the manufacturer's instructions. Flow

cytometry was performed using a FACScan ™ and data analysed using FloJOv6.1.2®. Sample identities were blinded to the assessor.

Statistical Analysis

Statistical analyses were performed using SPSS© 11th edition software. Values for WBLogTREC/ml were initially log transformed to allow parameteric testing. Population distributions were tested for normality with a one sample Kolmogorov-Smirnov test. Correlations for normally distributed data were tested for significance using Pearson's correlation coefficient. Differences between groups were assessed by analysis of covariance (ANCOVA) where other covariates were present, unless otherwise stated. Bonferroni corrections were applied to correct for multiple comparisons. Results were considered significant when $p \leq 0.05$.

Results

Thymic function declines with age throughout childhood and does not differ between JIA patients and healthy controls

WBLogTREC/ml was quantified in 70 patients with JIA and 110 healthy controls (HC). Table 1 describes the cohort. One additional patient had DiGeorge Syndrome (chromosome 22q11 deletion associated syndrome with thymic hypoplasia / aplasia) and a co-existing inflammatory arthritis. Figure 1A shows that there was no significant difference in thymic function between the groups after adjusting for age and gender (see below). Negative correlations with age were detected for both groups ($p < 0.01$). The patient with DiGeorge Syndrome had markedly deficient thymic function as evidenced by a 1.5Log deficit in WBLogTREC/ml when compared with

age matched controls. There was no difference in thymic function when ILAR JIA subtypes were compared (Figure 2A).

	N	Age / yrs - median (range)	Male (%)	DMARD any (%)	MTX (%)	Biologic* (%)	Oral steroid (%)	No DMARD**
Systemic Onset	8	10.52 (3.51-15.12)	6 (75)	7 (88)	5 (63)	3 (38)	3 (38)	1 (12.5)
Polyarticular RF+	10	9.92 (4.11-17.85)	3 (30)	7 (70)	6 (60)	1 (10)	3 (38)	2 (20)
Polyarticular RF-	12	12.59 (2.85-15.13)	2 (16)	7 (58)	6 (50)	2 (17)	3 (38)	5 (41)
Persistent oligoarticular	27	9.16 (2.06-28.74)	7 (27)	2 (7)	2 (7)	0	0	25 (93)
Extended oligoarticular	7	10.3 (4.03-17.96)	2 (28)	4 (57)	1 (14)	3 (43)	0	3 (43)
Psoriatic	4	19.3 (14.36-30.78)	3 (75)	2 (50)	1 (25)	1 (25)	1 (25)	2 (50)
Enthesitis	2	14.25 (12.8-15.69)	1 (50)	2 (100)	2 (100)	0	1 (50)	0

* Includes anti TNFα agents and anti IL-1. **Includes those taking only NSAIDs or relying only on local joint injections for disease control. RF – rheumatoid factor; DMARD disease modifying anti-rheumatic drug; MTX – methotrexate.

Table 1 – Patient characteristics according to ILAR subtype

The proportion of $CD4^+CD45RA^+CD31^+$ declines with age in HC and JIA

$CD4^+CD45RA^+CD31^+$ T-cells are TREC rich and have been proposed as the phenotype of RTE in humans (16). The proportion of T-cells with this phenotype (%RTE) declined with age in JIA (n = 50) and HC (n = 77) (Figure 1B) but there was no significant difference in age adjusted mean %RTE.

Since both WBLogTREC/ml and %$CD4^+CD45RA^+CD31^+$ T-cells have been proposed as markers of thymic function there should be a strong positive correlation between them, independent of disease status. Although the relationship was strong in

HC (r = 0.71; p < 0.01), it was less robust in JIA (0.45; p <0.01). There was no effect of ILAR subtype on %RTE (Figure 2B).

In our patient with DiGeorge Syndrome, the %RTE was normal despite a significantly reduced WBLogTREC/ml value (Figure 1B), suggesting that $CD4^+CD45RA^+CD31^+$ T-cells may be capable of self renewal in the absence of thymic replenishment.

Gender differences in thymic function are preserved in children with JIA

In both mice and adult humans, females have a larger thymic output (as determined by TREC quantification and histological analysis of thymic volume) than age matched males (20, 21). Using the two assays described above, we confirmed higher age-adjusted thymic output in females, both in HC and JIA patients (Figure 1 C & D). The age-adjusted mean WBLogTREC/ml value for HC females (n = 51) was 5.039 and for males (n = 59) 4.985 (p = 0.002); in JIA the comparable figures were 5.004 (n = 46) and 4.826 (n = 24), (p = 0.003).

Using %RTE a gender difference was again detected in HC (age adjusted mean %RTE in females (n = 36) 52.9%, males (n = 41) 45.6% (p = 0.001) but not in JIA group (data not shown).

Effect of immunosuppressant therapy on thymic function in JIA

Corticosteroids and some immunosuppressive treatments have been considered to inhibit thymic function (22-24) and we therefore sought an effect in JIA. It should be noted that the majority of children taking DMARDs or oral corticosteroids had more severe disease, falling into the systemic-onset, polyarticular and extended oligoarticular subtypes (see Table 1). However, when patients receiving these drugs were compared with those not receiving them and with healthy controls using

WBLogTREC/ml, no evidence of a significant effect of DMARDs (when considered collectively (n = 31)) (4.874 *v* 4.933 *v.* 4.985) (Figure 3A) or corticosteroids ($\pm$ DMARD) (n = 11) (4.818 *v.* 4.924 *v.* 4.985) was found (data not shown). There was, however, a trend toward lower WBLogTREC/ml values in JIA patients on a DMARD (p = 0.061) and those taking steroid ($\pm$ DMARD) (p = 0.07) when compared with healthy controls. There was no effect of taking MTX on WBLogTREC/ml values (4.887) when compared with those not on MTX (4.917) and with HC (4.985) (p = n.s) although some patients not on MTX were taking an alternative DMARD (primarily anti TNFα) (n = 8). Other treatment groups contained too few patients to exclude an independent effect. In contrast, after adjustment for age and gender, %RTE was significantly higher in patients on oral corticosteroid (n = 8; 58.3%) than in those not on oral corticosteroid (n = 40; 47.0%; p = 0.013) and the comparison with HC (n = 77) approached significance (49.8%; p = 0.067). There was no difference between those not on oral corticosteroid and HC. There was no significant effect of DMARD use (Figure 3B) or MTX specifically on %RTE.

Intra-thymic proliferation in JIA

When the TCRα chain gene rearranges, the locus encoding the TCRδ chain is excised in a conserved rearrangement resulting in the formation of a TREC that can be identified in approximately 70% of emerging thymocytes. This rearrangement is the basis of most standard TREC assays, including our own WBLogTREC/ml assay (17). When the TCRβ chain gene is rearranged, no single rearrangement occurs but there are only 13 potential D$_\beta$$\rightarrow$J$_\beta$ gene rearrangements (25). Dion *et al* devised an assay that quantifies 10 of these 13 TREC generating rearrangements, the sum of which provides an estimate of the frequency of T-cells in peripheral blood that have

rearranged their TCRβ chain gene ($\Sigma\beta$TREC) (19). TCRα chain rearrangement occurs after TCRβ chain rearrangement. Between these events a phase of thymocyte proliferation expands thymocytes with a rearranged TCRβ chain, providing a substrate for the subsequent generation of TCR diversity through α chain/β chain pairing. The ratio between the numbers of T-cells containing detectable TCRβ and TCRα chain rearrangements (αTREC:$\Sigma\beta$TREC) therefore gives an indication of the number of divisions, which is a measure of intra-thymic proliferation and overall thymic function.

We determined intra thymic function in JIA and HC. No difference was found when 19 children with JIA were compared with 19 healthy controls and there was also no detectable effect of gender (data not shown). There were insufficient samples to seek an effect of corticosteroid or DMARDs. The effect of ILAR sub classification was assessed. There were no significant differences between the groups after post hoc testing with the exception of all comparisons with the polyarticular RF$^+$ group (n = 3) which, (after the two groups where n = 1 were excluded), achieved statistical significance - Figure 4A. The true significance of this result is difficult to assess because of small patient numbers and was lost when we combined together the poor prognosis subtypes of systemic onset, polyarticular, and extended oligoarticular JIA ('non-oligoarticular', n = 9) and compared with persistent oligoarticular JIA ('oligoarticular' n = 11), a classification previously used (26) (Figure 4B).

In the patient with DiGeorge Syndrome, the αTREC:$\Sigma\beta$TREC ratio was 57 in comparison with the HC group median (352) and the JIA group median (628), reflecting the impaired structural environment of the DiGeorge thymus and its reduced capacity for thymocyte expansion. The $\Sigma\beta$TREC value in this patient was comparable to JIA values 1.58 *v.* 5.38 (range 0.53 − 17.41) whereas the αTREC value

was significantly reduced, 91 *v.* 3002 (range 851 − 5065). This implies that thymocyte proliferation is impaired in DiGeorge Syndrome in comparison with HC and children with JIA.

Thymus derived T_{Reg} are more frequent in persistent oligoarticular JIA

Lastly, we quantified the proportion of $CD4^+$ T-cells with the regulatory phenotype $CD4^+FOXP3^+$ (T_{Reg}). There was no detectable difference overall between JIA patients and HC. When individual ILAR subtypes were compared there was a weak but significant difference between the systemic and persistent oligoarticular disease patients (p = 0.031) in a post hoc analysis, although sample sizes were again small - Table 2. To strengthen our analysis by increasing group numbers, patients with 'non-oligoarticular' disease (as above) were compared with persistent oligoarticular patients. We found a significant increase in the proportion of T_{Reg} in the oligoarticular group (8.19 % *v.* 6.481%; p = 0.044 - Tukey HSD post hoc analysis), consistent with other reports. Unlike those reports however, there was no difference from HC. There was no effect of gender, age or DMARD use, although MTX use was more common in non-oligoarticular JIA (see Table 1), potentially confounding the difference between disease subtypes.

	n	%CD4$^+$FOXP3$^+$ (mean)	95%CI
Non oligoarticular§	27	6.48	5.54 -7.43
Systemic*	4	4.79	2.31 - 7.27
Polyarticular RF+	7	7.32	5.44 - 9.20
Polyarticular RF-	10	6.57	5.00 - 8.14
Extended Oligoarticular	6	6.49	4.45 - 8.51
Persistent Oligoarticular§,*	22	8.19	7.00 – 9.39
§Healthy controls	33	6.91	6.05 - 7.78

Table 2 Mean values for %CD4$^+$FOXP3$^+$ and 95% CI for the 'non-oligarticular' group (comprising systemic, polyarticular RF$^+$ and RF$^-$ and extended oligoarticular ILAR categories), the persistent oligoarticular group and a healthy control group.

§T$_{Reg}$ were significantly higher in the persistent oligoarticular group when compared with the non oligoarticular group ($p = 0.044$) but not healthy controls ($p = 0.720$).

When individual ILAR subtypes were compared there was a significant difference between the systemic and persistent oligoarticular ILAR subtypes ($p = 0.031$) - Games-Howell post-hoc test analysis (which accounts for the differing group sizes).

Discussion

Our data demonstrate that, in children with JIA, thymic function is comparable with that of healthy control children. This conclusion is strengthened by our analysis of multiple aspects of thymic function, including intra-thymic proliferation and 'down-stream' effects on recent thymic emigrants and T$_{Reg}$. Consistent with previous reports we have demonstrated greater thymic function in females (27) and an age-associated decline in healthy controls (28), findings that persist in JIA. In a single patient with inflammatory arthritis and co-existent DiGeorge syndrome we showed a dramatic reduction in thymic function, consistent with thymic hypo/aplasia and similar to the effect of partial thymectomy during paediatric cardiac surgery (29). Although we have previously confirmed that consecutive recruitment of patients from our clinic

population provides a group of patients with a typical case mix (30) (Table 1), the short recruitment period (4 months) may have biased our population toward those with more severe disease (since they are more likely to attend hospital).

When data were analysed according to ILAR disease subtype, a minority of comparisons reached statistical significance or demonstrated a trend in that direction. For example, there was a trend toward reduced thymic function for those on a DMARD (although since disease group and DMARD use co-segregate, this analysis is confounded by ILAR subtype), and steroid use was associated with an increase in the %RTE. The αTREC:$\Sigma\beta$TREC ratio was increased in the ILAR polyarticular RF[+] group, whilst the persistent oligoarticular group had a significantly higher proportion of T_{Reg} than the ILAR systemic disease group, consistent with previous reports. These isolated observations should be interpreted with caution due to the small group numbers and require further analysis in a larger study in order to determine their true significance.

T-cells with the phenotype $CD4^+CD45RA^+CD31^+$ have significantly higher TREC content *per cell* than unselected $CD4^+$ T-cells (16) confirming this subset is enriched for true thymic emigrants. Furthermore, $CD4^+CD45RA^+CD31^+$ T-cell telomeres are longer and telomerase activity higher than in $CD4^+CD45RO^+CD31^+$ T-cells, suggesting a limited replicative history (31). This phenotype has been used to monitor thymic function longitudinally (32-34) and although inter-individual variation is high, values are stable longitudinally ((34) and AR Lorenzi & JD Isaacs unpublished results). Thus, it was interesting to note that, despite a ~ 1.5Log deficit in WBLogTREC/ml in a patient with DiGeorge Syndrome, the proportion of

CD4$^+$CD45RA$^+$CD31$^+$ T-cells was age appropriate. Furthermore, WBLogTREC/ml and %RTE did not always correlate well, particularly in disease, (lending support to the recent observation that CD4$^+$CD45RA$^+$CD31$^+$ T-cells can in part be maintained by peripheral homeostasis (35)) and thus may represent a less useful measure of thymic function in this setting. Naïve T-cells proliferate excessively in RA patients, possibly under cytokine stimulation (36). A similar phenomenon in JIA would weaken the correlation between CD4$^+$CD45RA$^+$CD31$^+$ T-cells and WBLogTREC/ml, as we have shown here.

Regardless of its indication, adults fare less well than children following ASCT (37). In part this reflects an inverse relationship between age and CD4$^+$ T-cell reconstitution (38, 39) but, in autoimmunity, disease-related thymic compromise may exacerbate the distinction. For example, RA patients remain lymphodepleted for years following ASCT (40) but disease usually relapses rapidly despite persisting lymphopenia. In contrast, JIA patients reconstitute more rapidly and, moreover, thymically-derived T$_{Reg}$ may contribute to long-term remission of symptoms (41). Thus, measures aimed at reversing age-associated thymic atrophy (currently in clinical trial (42, 43) could significantly improve the outcome of ASCT in adults with autoimmune diseases.

The normal thymus produces T cells that are self-tolerant with a polyclonal TCR repertoire. Age-related thymic involution results in a proportionally greater decline in thymocytes with rearranged TCRα genes than with TCRβ chain genes (19), with potential implications for repertoire diversity (44) and therefore the maintenance of self tolerance, responses to neoantigens and tumour surveillance (45). We determined

a ratio that reflected the number of cellular divisions between these two events. The similarity between patients (when collectively considered) and controls suggests this step in thymocyte development is intact in JIA. When individually considered however, there was a significant increase in the ratio for the polyarticular RF$^+$ patients. A single extreme value contributes to this effect but the remaining two individual values were also greater than for all other subtypes (Figure 4A) a consequence of both low βTREC and high αTREC values (data not shown). The significant contribution of the αTREC value to the ratio and the variance of this value in both JIA and HCs (Figure 1) should also be noted and duplication of our results is required before further conclusions about this difference can be drawn. If confirmed, however, this result would suggest enhanced intra-thymic proliferation in the JIA subtype that most closely resembles adult RA, in which there is evidence of impaired thymic function (3). TCR diversity and TREC are positively correlated (46) suggesting that the TCR repertoire in JIA is also likely to be maintained.

De Kleer *et al* distinguished two subsets of T$_{Reg}$ in JIA peripheral blood following ASCT (41). Expansion of mature, non depleted or grafted T$_{Reg}$ in the lymphopenic environment was followed 6-8 months later by the appearance of T$_{Reg}$ of recent thymic origin (which may have greater suppressive function than memory CD45RO$^+$ T$_{Reg}$ (47)), together replenishing the depleted pre-transplant T$_{Reg}$ pool (41). Although we did not distinguish T$_{Reg}$ subsets, we found a higher proportion of T$_{Reg}$ in persistent oligoarticular disease than in non-oligoarticular disease although we could not confirm a difference between either group and HC, possibly reflecting our use of FOXP3 to quantify T$_{Reg}$, rather than CD25^{bright} (41). We have previously shown that adults with self-limiting reactive arthritis have a higher proportion of T$_{Reg}$ than adults

with RA (48) and murine studies have demonstrated that Foxp3$^+$ T-cells protect against autoimmune disease and inflammation in vivo (49). Collectively, these data suggest that control of inflammatory disease may be associated with this subset of T-cells. Indeed, all 4 systemic JIA patients studied here had a very low proportion of T_{Reg} in peripheral blood. However, their otherwise normal thymic function suggests that their T_{Reg} deficit may reflect a failure of peripheral T_{Reg} homeostasis in progressive, chronic disease.

In conclusion, using a variety of measurements we have demonstrated age-appropriate thymic function in JIA patients. We found no clear influence of therapy on thymic function although DMARD use was more common in poor prognosis JIA patients and was associated with trends toward lower WBLogTREC/ml values. We also found similar T_{Reg} values in HC and JIA although JIA patients of better prognosis subtype had higher values. Our findings differ from those in adult patients with autoimmune disease, in whom thymic dysfunction has been implicated as a possible aetiological factor (3-6).

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Figure 1 - Thymic function is not significantly different in children with JIA (open circles, dashed regression line) and healthy controls (HC) (filled circles- solid regression line) when quantified using WBLogTREC/ml (A) and by the proportion of T-cells with the phenotype $CD4^+CD31^+CD45RA^+$ (%RTE) (B). WBLogTREC/ml was significantly negatively correlated with age (r = -0.762; p<0.001 for controls and r = 0.625; p<0.001 in JIA) as was %RTE (r = -0.656; p<0.001 and r = 0.491; p<0.001 respectively). In a single patient with co-existent DiGeorge Syndrome (grey triangle with dashed circle surround), TREC levels were markedly reduced but %RTE was age appropriate.

In both HC (C) and JIA (D), females (open circles, dashed regression lines) have greater thymic function than males (closed squares, solid regression line) using WBLogTREC/ml (p = 0.002 and p = 0.003 respectively).

Figure 2 - Thymic function does not significantly differ between ILAR JIA subtypes when measured using either WBLogTREC/ml (A) or %RTE − (p = n.s) (B). Bars represent age adjusted group means ± SEM. Groups were compared using ANCOVA with age as a covariate and multiple comparisons corrected for by performing a Bonferroni correction.

(PolyRF − polyarticular RF positive and negative respectively, PsA − psoriatic, Enth − enthesitis associated, Ext oligo − extended oligoarticular, Persis oligo − persistent oligoarticular).

Figure 3 - There was no significant effect on thymic function when measured using WBLogTREC/ml (A) or %RTE (B) of being on a DMARD. Bars represent age and gender adjusted group means ± SEM. Groups were compared by ANCOVA with a Bonferroni correction to adjust for multiple comparisons. Patients not on a DMARD were more likely to have persistent oligoarticular disease than those taking one − see

Table 1. There was a trend toward lower WBLogTREC/ml values in those taking a DMARD (p = 0.061) when compared with healthy controls (HC). *Includes those taking only NSAIDs or relying only on local joint injections for disease control. **DMARDs include anti-TNFα agents and IL-1ra.

Figure 4A - The αTREC:ΣβTREC ratio (y axis) was compared between ILAR subtypes using ANCOVA with Bonferroni correction for multiple comparisons with age and gender as covariates because of their effect on the αTREC value. When the two subsets where n = 1 were excluded, the comparisons between the polyarticular RF$^+$ group and the reminder were statistically significant (p<0.05) - although caution should be applied to the interpretation of this result given the small group numbers. (Sys – systemic (n =3); PRF$^+$ & PRF$^-$ - polyarticular RF$^+$ / PRF$^-$ (n = 3 both groups); PsA – psoriatic (n =1); ExO– extended oligoarticular (n = 1); PerO - persistent oligoarticular (n = 8).

(B) When grouped into persistent oligoarticular disease and non-oligoarticular disease (see text) there were no significant differences between the groups. Boxes represent the inter-quartile ranges (IQR) and bars the group medians. Whiskers give the data range with '•' representing extreme values (> 3 IQR from the box end).