

## EXTENDED REPORT

# Differential expression patterns of recombination-activating genes in individual mature B cells in juvenile idiopathic arthritis

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**Background:** Re-expression of the recombination-activating genes (*RAG*) in peripheral B cells may be relevant in the development of autoreactive antibodies in autoimmune diseases. The presence of antinuclear antibodies (ANA) as a hallmark of oligoarticular juvenile idiopathic arthritis (o-JIA, early-onset type) indicates a breakdown in immunological tolerance.

**Aim:** To examine the expression of *RAG* genes in peripheral blood mature B lymphocytes in patients with o-JIA.

**Methods:** 777 memory B cells from peripheral blood, CD19+ CD27+ CD5+ or CD19+ CD27+ CD5−, isolated from three ANA+ children with o-JIA and three healthy age-matched children, were examined for the expression of *RAG1* and *RAG2* mRNA. mRNA transcripts of activation-induced cytidine deaminase and immunoglobulin G were searched to further determine their developmental stage.

**Results:** mRNA was present for any of the two *RAG* genes in the B cells of children with JIA and controls. However, the predominance of *RAG1* or *RAG2* was different. A significantly decreased frequency of *RAG2*-expressing memory B cells in both CD5+ and CD5− populations was noted in children with JIA ( $p < 0.001$ ), whereas the number of *RAG1*-expressing B cells was slightly increased. The coordinate expression of both the *RAG* genes was a rare event, similar in the CD5+ populations (1% in controls, 2% in children with JIA), but different among the CD5− compartments (5% v 0%;  $p < 0.01$ ).

**Conclusion:** These results argue for a reduced coordinate *RAG* expression in the peripheral CD5− memory B cells of patients with o-JIA. Thus, it was hypothesised that impaired receptor revision contributes to autoimmune pathogenesis in JIA.

Oligoarticular juvenile idiopathic arthritis (o-JIA)<sup>1</sup> is one of the frequently diagnosed subtypes and is associated with human leucocyte antigen alleles and antinuclear antibodies (ANA). The contribution of ANA to the pathogenesis of JIA has been investigated poorly. As in other autoimmune diseases such as systemic lupus erythematosus (SLE) or rheumatoid arthritis, the detection of autoreactive antibodies is clinically relevant for making the diagnosis. High levels of rheumatoid factor or ANA in patients with rheumatoid arthritis or SLE suggest a pathogenetic relevance. The reason why tolerance is broken and why these antibodies are produced in JIA remains unknown.

During their early development in the bone marrow, lymphocytes undergo rearrangements of their genomic immunoglobulin loci to diversify their antibody repertoire. In this process, *RAG* proteins, as transcripts of the recombination-activating genes 1 (*RAG1*) and 2 (*RAG2*), have a crucial role. Their expression is strictly limited to lymphocytes. The process of variable, diversity and joining gene segments (V(D)J) recombination results in the production of functional surface immunoglobulins. A mature B cell compartment cannot be established when *RAG* genes are defective.<sup>2–4</sup>

V(D)J rearrangement has long been considered to be restricted to early B cell precursors in the bone marrow. However, it has been shown that in the immature bone marrow stages and even outside the bone marrow micro-environment, secondary rearrangements occur to rescue lymphocytes with self-reactive antibodies from negative selection. This salvage pathway was termed receptor editing in bone marrow and receptor revision in the periphery.<sup>5</sup> Until now, the role of receptor revision in tolerance induction has

not been fully established. Evidence shows that pathological activation of receptor revision can even lead to a breakdown in tolerance. Recently, mature B cells in germinal centres have been shown to undergo receptor revision after exposure to interleukin (IL)4 and lipopolysaccharide or CD40L and after immunisation.<sup>6–9</sup> Others have shown *RAG* reinduction in circulating mature peripheral B cells on stimulation with *Staphylococcus aureus* Cowan 1 and IL2.<sup>10</sup> Receptor revision in mature peripheral B cells, however, has been challenged by reports stating that *RAG*-expressing peripheral lymphocytes in part exhibited an immature phenotype. Such an expression of VpreB, IL7R and TdT may result from a premature release from the bone marrow or the presence of transitional B cells.<sup>11–14</sup>

Secondary rearrangements may have an important role in autoimmune diseases. Two different mechanisms can be envisaged. Disease may be promoted by an uncontrolled creation of autoreactive antibodies in the periphery. On the other hand, receptor revision may rescue B cells that have acquired autoreactive receptors.<sup>15–18</sup> In addition, a deficiency in receptor editing or receptor revision may be a cause for ineffective deletion of autoreactive B cells as proposed for SLE.<sup>19 20</sup>

CD5+ B cells, part of the B1 cell compartment, are suggested to have a major role in innate immunity and are found predominantly in body cavities. They produce mainly

**Abbreviations:** AID, activation-induced cytidine deaminase; ANA, antinuclear antibodies; dUTP, deoxyuridine triphosphate; o-JIA, oligoarticular juvenile idiopathic arthritis; PCR, polymerase chain reaction; *RAG*, recombination-activating gene; SLE, systemic lupus erythematosus

**Table 1** Patient details at the time of sampling

Patient number	1	2	3
Age (years)	5	3	4
Active joints (n)	0	1	1
ESR (mm at the end of the first hour)	7	14	20
Hb (g/dl)	12.5	11.8	12.8
Thr (per $\mu$ l)	306 000	349 000	421 000
ANA	1:640	1:640	1:2.560
Drugs	–	Naproxen	Naproxen, methotrexate
Duration of disease (months)	37	4	35

ANA, antinuclear antibodies; ESR, erythrocyte sedimentation rate; Hb, haemoglobin; Thr, thrombocytes.

polyreactive antibodies of the immunoglobulin (Ig)M class, with a restricted repertoire. Owing to their polyreactivity, these cells have the potential to contribute to autoimmunity. This has been postulated in several reports showing raised numbers of this B cell subset in systemic autoimmune diseases,<sup>21</sup> including JIA.<sup>22–23</sup>

To assess whether human memory B lymphocytes as defined by the expression of surface CD27<sup>24</sup> exhibit an irregular expression of RAG genes in an autoimmune context, we examined peripheral blood CD19+ CD27+ B lymphocytes from healthy children and ANA-positive patients with o-JIA. With a sensitive single-cell reverse transcriptase-polymerase chain reaction (PCR) technique, the expression of RAG1 and RAG2 was evaluated in individual CD19+ CD27+ CD5+ or CD19+ CD27+ CD5– B cells. We also searched for transcripts of IgG and activation-induced cytidine deaminase (AID) as markers for germinal centre B cells.<sup>25–26</sup>

## PATIENTS, MATERIALS AND METHODS

### Patients

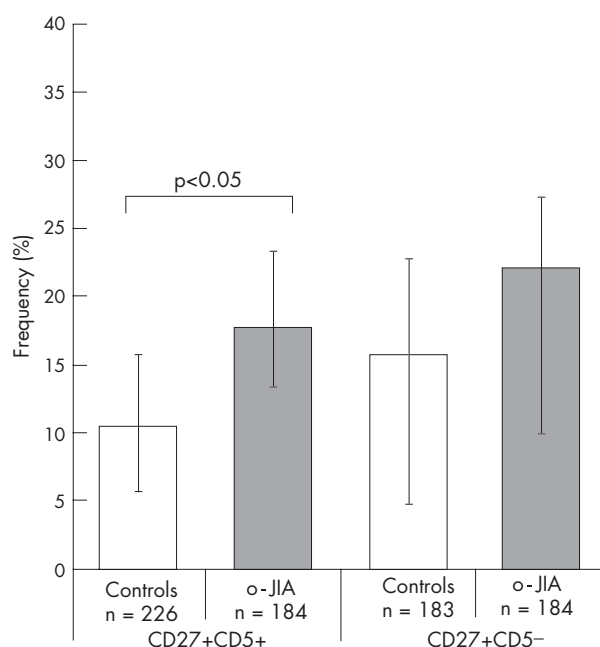
For the examination of individual B cells we took heparinised blood samples from three ANA-positive paediatric patients diagnosed with persistent o-JIA.<sup>1</sup> Three age-matched healthy children served as controls. The mean (range) age for children with o-JIA was 4.6 (3–5) years and for controls was 4.7 (2–8) years (table 1). Parents gave informed consent. The study was conducted according to the modified Declaration of Helsinki and the ethics committee of the University of Würzburg approved the study.

### Preparation of B cells from tonsil tissue for detection of AID mRNA

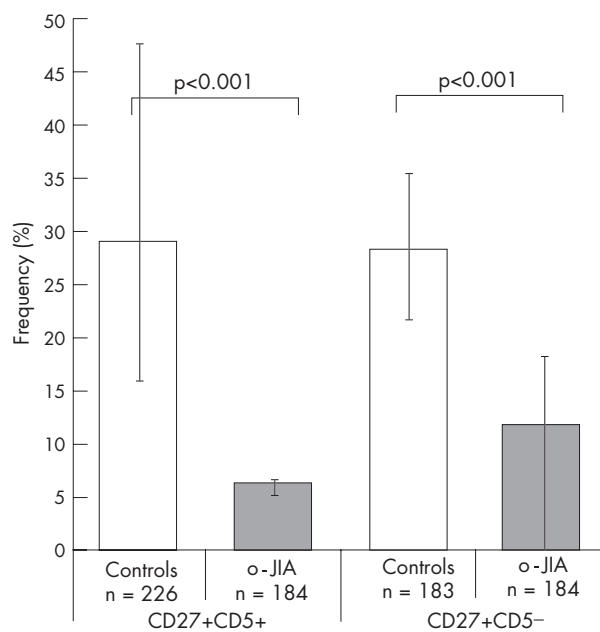
A tonsil from a healthy child was obtained after tonsillectomy. Suspensions of tonsillar mononuclear cells were prepared by collagenase digestion (Worthington Biochemical, Lakewood, New Jersey, USA) of the tissue for 30 min, followed by Ficoll–Hypaque density gradient centrifugation.<sup>7</sup> Subsequently, cells were stained with anti-CD19 (isothiocyanate-labelled, Caltag, Burlingame, California, USA), anti-IgD (fluorescein isothiocyanate-labelled, Caltag) and anti-CD38 (PE-labelled, BD Pharmingen, San Diego, California, USA), and sorted.

### Preparation of B cells from peripheral blood

Peripheral blood mononuclear cells from heparinised blood samples were separated by the Ficoll–Hypaque density gradient. For single-cell sorting, cells were three-colour stained and incubated for 20 min with anti-CD19 (isothiocyanate-labelled, Caltag), anti-CD27 (fluorescein isothiocyanate-labelled, BD Pharmingen) and anti-CD5 (PE-labelled,



**Figure 1** Frequencies of memory B cells expressing recombination-activating gene 1 (RAG1) mRNA in children with oligoarticular juvenile idiopathic arthritis (o-JIA). Individual peripheral B cells isolated from three children with o-JIA and three age-matched healthy children bearing the indicated phenotype (CD19+ CD27+ CD5+ or CD19+ CD27+ CD5–) were sorted by fluorescence-activated cell sorter. Only  $\beta$ -actin-positive (n) B cells were analysed. The frequency of cells expressing RAG1 mRNA in the total cohort was determined by single-cell polymerase chain reaction and is indicated on the y axis. The bars indicate the range among individual patients.



**Figure 2** Frequencies of memory B cells expressing recombination-activating gene 2 (RAG2) mRNA in children with oligoarticular juvenile idiopathic arthritis (o-JIA). Individual peripheral B cells isolated from three children with o-JIA and three age-matched healthy children bearing the indicated phenotype (CD19+ CD27+ CD5+ or CD19+ CD27+ CD5–) were sorted by fluorescence-activated cell sorter. Only  $\beta$ -actin-positive (n) B cells were analysed. The frequency of cells expressing RAG2 mRNA in the total cohort was determined by single-cell polymerase chain reaction and is indicated on the y axis. The bars indicate the range among individual patients.

Caltag) antibodies, followed by two washing steps. Isotype-matched antibodies served as controls.

### Single-cell sorting

Using a FACSVantage Flow Cytometer (Becton Dickinson, San Diego, California, USA) equipped with a single-cell deposition unit, the two populations of CD19+ CD27+ CD5+ and CD19+ CD27+ CD5- cells were identified and individual cells from each population were deposited in 96-well PCR plates, as described previously.<sup>7,17</sup> Tonsillar IgD+ CD38++ B cells were sorted as individual B cells to examine *AID* gene expression.

### Preparation of RNA and cDNA from sorted single cells

Before sorting cells into 96-well plates, each well was provided with 10  $\mu$ l of lysis solution (4.85  $\mu$ l aqua, 2  $\mu$ l 5 $\times$  first-strand buffer (Invitrogen, Karlsruhe, Germany); 1  $\mu$ l 0.1 M dithiothreitol (Invitrogen); 1  $\mu$ l 1% Nonidet-NP40 (Sigma, St Louis, Missouri, USA); 10 units of RNasin ribonuclease inhibitor (Promega, Madison, Wisconsin, USA); 0.8  $\mu$ l 10 mM deoxynucleotide triphosphate (dNTP; Sigma); 0.1  $\mu$ g oligo-(dT)<sub>12-18</sub> (Amersham Pharmacia Biotec, Piscataway, New Jersey, USA). RNA to cDNA was reverse transcribed as described previously by using Superscript II RNase H-RT (Invitrogen).<sup>7,17</sup> cDNA was stored at -72°C before further processing.

### External and nested PCR amplification

*AID* was amplified at 94°C for 3 min, 36 cycles of 30 s at 94°C, 60 s at 60°C and 90 s at 72°C, followed by a final extension step of 3 min at 72°C. The following primers (5'-3') were used:

- External, sense: GAGGCAAGAAGACTCTGG<sup>27</sup>
- External, antisense: GTGACATTCTGGAAGTTGC<sup>27</sup>
- Nested, sense: TACTTCTGTGAGGACCGCAA
- Nested, antisense: CACACAGGGGCAAAAAGGATG
- Oligonucleotide ACTTTCAAAGCCTGGGAAGG used for detection.

IgG transcripts of  $\beta$ -actin, *RAG1*, *RAG2* exon 2a, *RAG2* exon 2b and IgG were amplified under the same conditions using the same oligonucleotides as described earlier.<sup>7,17</sup> In brief, 1.5  $\mu$ l of cDNA was used to carry out external PCR for each gene, followed by 5  $\mu$ l from external product for the nested PCR. Wells without reverse transcriptase and without cDNA served as negative controls and did not yield a product. cDNA from tonsil extracts was used as a positive control. The exclusion of genomic DNA amplification was carried out as described previously.<sup>7,17</sup>

### Detection of amplified cDNA by Southern blotting

Nested PCR products were transferred to a nylon membrane (ZetaProbe, BioRad, Hercules, California, USA) by Alkaline Dot-Blot (BioRad), followed by DNA crosslinking under the influence of ultraviolet light. Membranes were then incubated in a hybridisation buffer containing DIG-deoxyuridine triphosphate (dUTP)-labelled (Roche, Mannheim, Germany) oligonucleotides specific for the amplified sequences. DIG-dUTP was later detected by the chemoluminescent reaction, using alkaline phosphatase-coupled anti-DIG-dUTP antibody and CSPD (Roche). Results were visualised by exposure to photographic film. Blots were analysed on a digital detection unit (BioRad) using QuantityOne Software (BioRad).

### Statistical analysis

The  $\chi^2$  test and Fisher's exact test were used to analyse statistical differences between the total of single cells from three patients and three controls. Levels of significance

and high significance were set at  $p < 0.05$  and  $p < 0.01$ , respectively. Only B cells positive for  $\beta$ -actin were considered for analysis.

## RESULTS

### Expression of RAG genes and IgG mRNA in CD27+ CD5+ B cells

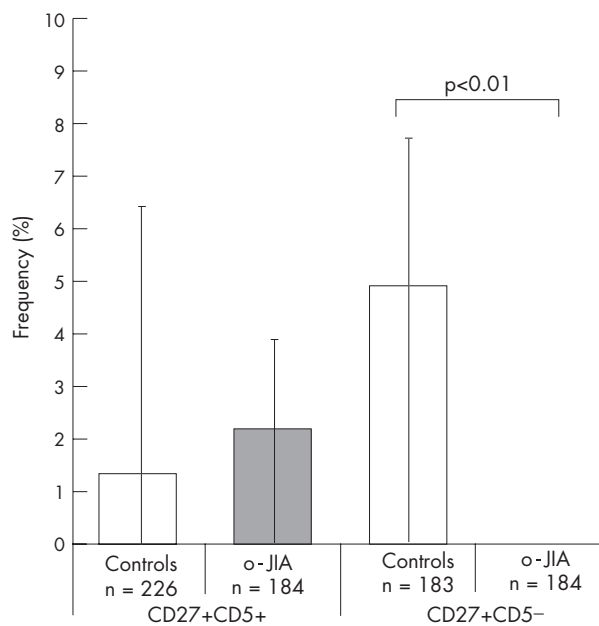
When we compared 226 B cells isolated from controls and 184 from patients with o-JIA, we found a low frequency of *RAG1*-expressing cells in controls and a higher number in patients with o-JIA (mean 11% v 18%;  $p < 0.05$ ; fig 1). Examination for *RAG2* mRNA expression showed a mean of 29% positive cells in controls, whereas lower levels were seen in patients with JIA (mean 6.5%;  $p < 0.001$ ; fig 2). Transcripts of either of the *RAG* genes were detectable to a considerable extent in each donor sample from both healthy children and children with JIA (mean 37% v 20%), with a significant reduction in transcripts from children with JIA ( $p < 0.001$ ). This was mainly attributed to the low *RAG2* expression.

A coordinate expression of both *RAG1* and *RAG2* in individual cells was found only in a few cells in this B cell population, irrespective of whether controls or patients were analysed (1% in controls, 2% in patients with o-JIA; fig 3).

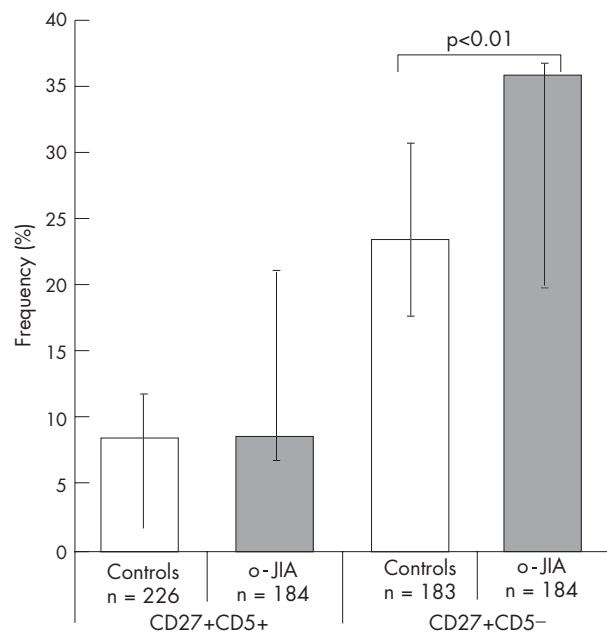
The frequency of CD27+ CD5+ B cells expressing IgG mRNA was 8% and 9% among patients and controls, and the difference was not significant (fig 4). None of the *RAG* double-positive cells was positive for IgG mRNA in controls or in patients with o-JIA (table 2).

### Expression of RAG genes and IgG mRNA in CD27+ CD5- B cells

In the CD27+ CD5- population, we examined 183 single B cells isolated from controls and 184 cells from children with o-JIA. *RAG1* expression showed a comparable distribution as



**Figure 3** Frequencies of memory B cells expressing recombination-activating genes 1 and 2 (*RAG1* and *RAG2*) mRNA isolated from children with oligoarticular juvenile idiopathic arthritis (o-JIA). Individual peripheral B cells isolated from three children with o-JIA and three age-matched healthy children bearing the indicated phenotype (CD19+ CD27+ CD5+ or CD19+ CD27+ CD5-) were sorted by fluorescence-activated cell sorter. Only  $\beta$ -actin-positive (n) B cells were analysed. The frequency of cells expressing *RAG1* mRNA in the total cohort was determined by single-cell polymerase chain reaction and is indicated on the y axis. The bars indicate the range among individual patients.



**Figure 4** Frequencies of immunoglobulin G mRNA expressing CD27+ memory B cells isolated from children with oligoarticular juvenile idiopathic arthritis (o-JIA). Individual peripheral B cells isolated from three children with o-JIA and three age-matched healthy children bearing the indicated phenotype (CD19+ CD27+ CD5+ or CD19+ CD27+ CD5-) were sorted by fluorescence-activated cell sorter. Only  $\beta$ -actin-positive (n) B cells were analysed. The frequency of cells expressing RAG1 mRNA in the total cohort was determined by single-cell polymerase chain reaction and is indicated on the y axis. The bars indicate the range among individual patients.

already seen in the CD5+ subset, with an increased number among patients with JIA compared with controls (mean 22% v 16%;  $p = 0.12$ ; fig 1). RAG2 expression was also found to be similar when compared with the CD5+ population (mean 28% v 12%;  $p < 0.001$ ; fig 2).

Although patterns of individual RAG1 or RAG2 expression were comparable, the coordinate expression of RAG1 and RAG2 was strikingly different. In both healthy children and in those with o-JIA, 34% of all CD5- B cells expressed either of the RAG genes. A coordinate expression of RAG1 and RAG2 was seen in 5% of B cells from controls (9/183; ie, 7.2%, 7.7% and 0.0%), but not in those from children with o-JIA ( $p < 0.01$ ; fig 3).

When the expression of IgG in the CD5- population was studied, we found levels of IgG-producing B cells higher than in the CD5+ population. Particularly, a larger frequency was found in children with o-JIA than in controls (mean 36% v

23%;  $p < 0.01$ ). These results are consistent with phenotypical data from peripheral CD27+ B cells, where surface IgG ranged between 22% and 55%.<sup>27</sup>

In controls, RAG1-expressing cells were significantly over-represented in the IgG mRNA-positive (13/43, 30%) compared with the IgG mRNA-negative compartment (16/140, 11%;  $p < 0.01$ ; table 2).

### Expression of AID mRNA in peripheral B cells isolated from children with o-JIA

To further analyse the phenotype of CD27+ B cells and to exclude the possibility of them having recently been washed out of germinal centres in the course of a general inflammation, we also searched for transcripts of AID as a germinal centre marker in individual CD27+ CD5+ and CD27+ CD5- B cells isolated from one of the patients with o-JIA (patient 2). None among 76 (CD27+ CD5+) and 87 (CD27+ CD5-) B cells yielded a PCR product. The method was validated by carrying out PCR with the setting on individual IgD+ CD38+ germinal centre founder cells from a human tonsil, where transcripts of AID were detectable in 6/36  $\beta$ -actin-positive cells.

### DISCUSSION

We examined mature CD27+ peripheral blood B lymphocytes for the expression of RAG genes on a single-cell level. RAG gene expression is detectable in mature B cells. About one third of 777 B lymphocytes examined from all populations together had detectable levels of any of the RAG genes. Our results challenge recent views that RAG1 and RAG2 expression physiologically occurs only in a coordinate 1:1 relationship.<sup>28</sup> This hypothesis, however, has been generated in the analysis of "primary" V(D)J recombination of early B cell progenitors in bone marrow. Recently, evidence is emerging that receptor revision in the periphery may be an important process that ensures tolerance.<sup>16, 29</sup> Our findings give new insights into divergent RAG1 and RAG2 expression, which are partly supported by findings that engagement of the BCR can have contrary effects in distinct developmental stages of B lymphocytes.<sup>30</sup>

We found a more than twofold and fourfold decrease in the frequency of RAG2-expressing B cells isolated from patients with o-JIA in CD27+ CD5+ and CD27+ CD5- populations when compared with healthy people. In contrast, RAG1 frequencies tended to be unchanged or slightly increased in those with o-JIA. Thus, we hypothesised that RAG2 is differentially expressed in patients with o-JIA when compared with healthy people. Finally, this resulted in a loss of coordinate RAG expression in CD5- but not in CD5+ B cells.

The argument that an increased bone marrow lymphopoiesis results in a major fraction of immature RAG-expressing B cells in the periphery during a systemic inflammatory

**Table 2** Fractions of IgG-positive and IgG-negative B cells that are positive for RAG genes

	Healthy		o-JIA	
	IgG pos	IgG neg	IgG pos	IgG neg
CD19+ CD27+ CD5+ (n)	19	207	16	168
RAG1 (%)	11	11	13	18
RAG2 (%)	32	29	0	7
RAG1+RAG2 (%)	0	1	0	2
CD19+CD27+CD5- (n)	43	140	66	118
RAG1 (%)	30 ( $p < 0.01$ )	11	23	22
RAG2 (%)	33	27	14	11
RAG1+RAG2 (%)	9	4	0	0

Ig, immunoglobulin, neg, negative; o-JIA, oligoarticular juvenile idiopathic arthritis; pos, positive; RAG, recombination-activating gene.



process<sup>14</sup> is not applicable to our results, as we studied CD27+ mature B cells. Arguably, the effects seen are caused by cell cycle dependence of *RAG* expression, as a larger fraction of proliferating lymphocytes can be assumed in inflammation. Relative percentages of CD27+ among CD19+ cells were comparable in patients with o-JIA and in controls in a current flow-cytometric analysis, with a mean of about 16–18% (data not shown). No evidence in our analysis and in previous publications<sup>23</sup> showed an increase in total B cells isolated from patients with o-JIA. Thus, considerably differing absolute CD27 counts are unlikely. Although the *RAG2* protein is down regulated at least 20-fold before a cell enters the S phase to protect against unselective recombination under vulnerable conditions,<sup>31, 32</sup> these changes in the *RAG* protein level occur primarily at a post-translational level, with *RAG2* mRNA levels remaining nearly unaffected.<sup>31</sup> So far, it cannot be excluded that drugs had an effect on the composition of lymphocyte subsets, as we have previously shown for cyclophosphamide in SLE in children.<sup>33</sup> However, the completely missing coordinate *RAG* expression in CD5– B cells isolated from patients with JIA, despite a broad spectrum in disease activity compared with a solid presence in two of three in controls, would argue for a disease-related phenomenon.

Our results from human peripheral CD27+ B cells are concordant with recent reports on individual tonsil B cells from healthy children, in whom distinct developmental stages of germinal centre B cells were examined for *RAG* gene expression, with a special focus on CD5 expression<sup>29</sup>: on CD38+ IgD+ CD23+ B cells, induction of surface CD5 after stimulation with anti-IgM and CD40L was described, followed by coordinate *RAG* mRNA expression. Interestingly, *RAG2* transcripts appeared later than *RAG1* transcripts, supporting the hypothesis of a differential regulation of these two genes, and of *RAG2* as a limiting factor.<sup>29</sup> In contrast, among early tonsil memory B cells (CD38– IgD–), nearly three times as many double-*RAG*-positive cells were found in the CD5– subgroup as in the CD5+ population (16.6 v 6%). These findings are comparable to our results in peripheral CD19+ CD27+ B cells of controls: the relationship was similar, with 4.9% and 1.3% in CD5– and CD5+ B cells, respectively ( $p < 0.05$ ). Interestingly, the absolute frequencies of cells expressing *RAG1* and *RAG2* were lower in our study on peripheral B cells. Thus, a small subset of B cells expressing *RAG1* and *RAG2* seems to persist physiologically in the peripheral blood of children, as we have described previously in the case of adults.<sup>7</sup> Reduced coordinate *RAG* expression in CD27+ CD5– B cells isolated from patients with o-JIA may be relevant in pathogenesis, possibly disabling these B cells to carry out sufficient receptor revision.

The regulation of *RAG* genes is complex and tightly regulated, owing to the possible disastrous consequences of unselective DNA cleavage. It can happen at the level of transcription, post-transcriptional mRNA and protein modification or DNA accessibility (reviewed by Schlissel<sup>34</sup>). It has been difficult so far to assess factors influencing *RAG* expression: it is known that BCR engagement itself can turn off the expression of V(D)J recombinase genes in mature B cells.<sup>35, 36</sup> *RAG* can be induced again in these cells via a combination of CD40L or lipopolysaccharide and IL4 (or IL7) signalling, imitating T cell help.<sup>9, 37</sup> In contrast, in immature B cells, the opposite reaction has been documented. *RAG* genes have been induced after BCR engagement.<sup>38</sup>

Among CD5+ B cells, low coordinate *RAG* transcripts were detected in patients and in controls. In the CD5– children with JIA, we found no coordinate *RAG* expression at all. The CD5 molecule negatively regulates antigen receptor-mediated growth signals by recruiting SH2-domain-containing protein

tyrosine phosphatase-1 into the BCR.<sup>39</sup> Thus, CD5 action may have a stimulating role on the final coordinate *RAG* expression in patients with JIA by inhibiting a BCR-mediated suppression of *RAG* transcription in those with JIA. This may be due to a strong challenge of autoantigens via the BCR, which, on the other hand, suppresses *RAG* transcription, especially in B cells lacking the CD5 molecule in patients with JIA, as shown in our analysis. Thus, we hypothesise that pathogenetic processes are imposed on mature B cells, which become engaged in autoimmunity.

In earlier studies, we have shown increased coordinate *RAG* expression in IgD+ B cells from peripheral blood of patients with SLE,<sup>17, 33</sup> proposing a prolonged receptor editing in B cells from patients with SLE, which have prematurely left the bone marrow. As these cells represent an earlier stage in B cell development, comparability to the currently analysed post-switch memory B cells in patients with JIA is limited.

Indeed, these findings may show two different mechanisms proposed for the role of secondary rearrangements in the pathogenesis of autoimmune diseases, both promoting the disease by an uncontrolled creation of autoreactive antibodies. In contrast, an impaired receptor revision may result in ineffective deletion of B cells that have acquired autoreactive receptors in patients with o-JIA. However, the consequences of these immunoregulatory abnormalities on the pathogenesis of autoimmune diseases cannot be defined yet.

In conclusion, we found comparable low frequencies of *RAG* double-positive CD27+ B cells in healthy children and CD5+ populations with JIA, whereas the CD5– populations showed a complete absence of *RAG1*-positive and *RAG2*-positive lymphocytes in peripheral memory B cells in children with o-JIA. We obtained evidence for a lack of receptor revision in the periphery as a feature for CD27+ CD5– B cells in children with JIA, which may contribute to the autoimmune pathogenesis of the disease.

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Competing interests: None.

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