

INCREASE IN CD5+ B CELLS IN JUVENILE RHEUMATOID ARTHRITIS

Relationship to IgM Rheumatoid Factor Expression and Disease Activity

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Objective. To investigate the association between CD5+ B cell expression and IgM rheumatoid factor (IgM-RF) in juvenile rheumatoid arthritis (JRA).

Methods. CD5+ B cell levels analyzed by flow cytometry and IgM-RF expression determined by enzyme-linked immunosorbent assay were compared in children with JRA, children with other collagen vascular diseases, and healthy controls.

Results. Children with polyarticular JRA had expanded populations of CD5+ B cells, and expansion of CD5+ B cells and IgM-RF both correlated with disease activity.

Conclusion. The results indicate that an expanded CD5+ B cell population leads to IgM-RF production in patients with polyarticular JRA, as well as patients with RA.

The presence of IgM rheumatoid factors (IgM-RF) is a well-described feature of juvenile rheumatoid arthritis (JRA) (1). IgM-RF expression appears to

correlate with disease activity in JRA (1,2), suggesting that IgM-RF plays a role in the pathogenesis of the disease.

The main sources of IgM-RF are B cells that express the pan-T cell marker CD5 (Leu-1) (3,4). Expanded populations of these cells have been described in adult RA (5). Whether the expression of CD5 on B cells is a marker for B cell activation or identifies a distinct cell lineage remains controversial (5).

Although Martini et al have described elevated levels of CD5+ lymphocytes in children with JRA, all of the children they studied were negative for IgM-RF (presumably by the latex agglutination method) (6). However, several investigators (7,8) have shown that IgM-RF are commonly found in children with JRA when sensitive enzyme-linked immunosorbent assay (ELISA) techniques are used. We conducted the present study to test the hypothesis that expanded populations of CD5+ B cells occur in JRA and are associated with IgM-RF detected by ELISA.

PATIENTS AND METHODS

Patients. The study subjects were children between the ages of 15 months and 10 years, who were attending the Rheumatology/Immunology Clinic at the Children's Hospital of Michigan. Sixteen of the children (mean \pm SD age 6 ± 3.3 years) had polyarticular JRA and 13 (age 3.7 ± 2.3 years) had pauciarticular JRA (9). Eight children (age 7.6 ± 3.4 years) with other collagen vascular diseases (5 with juvenile dermatomyositis and 1 each with systemic lupus erythematosus, ankylosing spondylitis, and uncharacterized myositis/retinitis) were also included. Control subjects included 7 children with nonspecific musculoskeletal complaints, 3 children referred for immunologic evaluation and subsequently shown to have normal immunologic functioning, and 13

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healthy children. The mean age of the control subjects was 4.2 ± 2.9 years.

All children studied had negative findings on latex agglutination tests for IgM-RF. All those with JRA were taking nonsteroidal antiinflammatory drugs at the time of study. In addition, 2 children with polyarticular disease were taking oral gold, at a dosage of 3.0 mg/day, and 2 children were receiving oral methotrexate, 5.0 mg weekly.

Disease activity. Children with polyarticular JRA were considered to have active disease if they presented with warmth and effusion and/or synovial thickening of more than 3 joints. Children with pauciarticular disease were considered to have active disease if they presented with detectable warmth and synovial thickening in at least 1 joint.

Antibodies. Monoclonal anti-CD5 conjugated with phycoerythrin (PE) and pan-B cell-specific monoclonal anti-CD20 conjugated with fluorescein isothiocyanate (FITC) were obtained from Coulter Immunology (Hiialeah, FL). Human IgG for the IgM-RF ELISA was obtained from Sigma (St. Louis, MO).

Dual-fluorescence flow cytometry. One hundred microliters of whole blood was incubated with $5.0 \mu\text{l}$ of PE-conjugated anti-CD5 and $5.0 \mu\text{l}$ of FITC-conjugated anti-CD20. Cells were washed 3 times in phosphate buffered saline (PBS) and erythrocytes were lysed using a whole blood lysing kit (Coulter). The cells were then washed twice, resuspended in PBS, and analyzed by flow microfluorometry on an EPICS C flow cytometer, to determine the percentage of red- and/or green-stained lymphocytes gated by forward-angle and 90° -angle light-scattering.

ELISA for IgM-RF. Serum from children with JRA or from controls was diluted 1:100, and the ELISA for IgM-RF was performed as described by Moore et al (7). Plates were read at 405 nm and results were compared with a standard curve derived from control sera run at the same time. Optical density readings >2 standard deviations from the mean in the control group were considered to represent IgM-RF positivity.

Statistical analysis. Differences between mean percentages of CD5+ B cells were assessed by analysis of variance, using Scheffe's F test or Fisher's PLSD test. Chi-square analysis was used to examine the relationship between disease activity and the presence or absence of IgM-RFs. *P* values less than 0.05 were considered significant.

RESULTS

A comparison of the percentage of CD5+ B cells in the different study groups is shown in Figure 1. The mean \pm SD percentage of CD5+ B cells was significantly greater in children with polyarticular JRA ($40.9 \pm 22\%$) than in children with other collagen vascular diseases ($20.0 \pm 9\%$) ($P = 0.0143$) or in healthy controls ($22.1 \pm 8\%$) ($P = 0.0028$). The level of circulating CD5+ B lymphocytes in children with pauciarticular JRA ($27.5 \pm 9\%$) was intermediate between that of the children with polyarticular disease and the 2 control groups. The difference in the mean

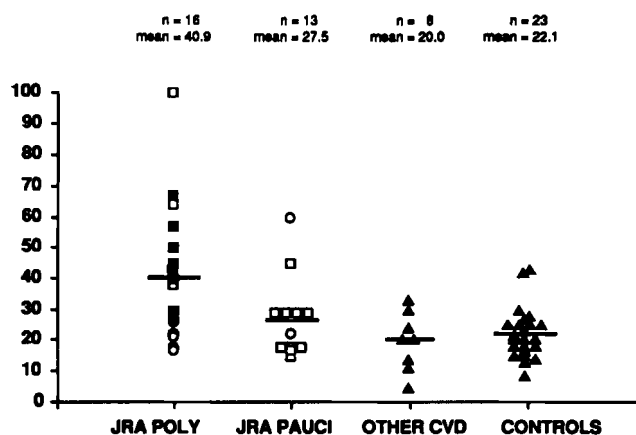


Figure 1. Percentages of B cells expressing CD5, as determined by dual-fluorescence flow cytometry, in patients with polyarticular juvenile rheumatoid arthritis (JRA POLY), patients with pauciarticular JRA (JRA PAUCI), patients with other collagen vascular diseases (CVD), and healthy controls. For the JRA patients, squares indicate active disease and circles indicate inactive disease; closed symbols indicate IgM rheumatoid factor (IgM-RF) positivity by enzyme-linked immunosorbent assay (ELISA) and open symbols indicate IgM-RF negativity by ELISA. The mean percentage of CD5+ B cells (represented by horizontal lines) was significantly greater in children who had polyarticular JRA compared with children who had other collagen vascular diseases ($P = 0.0143$), with healthy controls ($P = 0.0028$), and with children who had pauciarticular JRA ($P < 0.05$). Although the mean level of CD5+ B cells was higher in children with pauciarticular JRA than in the control groups, the differences were not statistically significant.

level among children with pauciarticular disease and that among children with polyarticular disease was also statistically significant ($P < 0.05$).

Elevated levels of CD5+ B cells in children with polyarticular JRA occurred only in those with active disease. All but 1 of the 10 children with active polyarticular JRA had $>30\%$ CD5+ B cells, whereas none of the 6 children with inactive polyarticular JRA had levels this great. The mean \pm SD level of CD5+ B cells in children with active polyarticular JRA ($53.0 \pm 20\%$) (Table 1) was more than twice the average level in children with inactive polyarticular JRA ($22.0 \pm 5\%$); the level in the latter group was similar to that found in the healthy subjects. In children with pauciarticular JRA, there was no demonstrable correlation between disease activity and CD5+ B cell levels.

IgM-RF was detected in 8 of the 16 children with polyarticular JRA, but was undetectable in any of the other children tested. Among the children with detectable IgM-RF, 7 of 8 had active disease. However, 3 of the 10 children with active disease did not have detectable IgM-RF, and all 3 of these children

Table 1. CD5+ B cell IgM rheumatoid factor (IgM-RF) expression, by disease activity, in patients with polyarticular and pauciarticular juvenile rheumatoid arthritis (JRA)

Group, disease status	% CD5+ B cells (mean \pm SD)	No. with >30% CD5+ B cells	No. with hidden RF
Polyarticular JRA			
Active	53 \pm 20	9/10	7/10
Inactive	22 \pm 5	0/6	1/6
Pauciarticular JRA			
Active	26 \pm 9	1/10	0/10
Inactive	33 \pm 23	1/3	0/3

had elevated levels of CD5+ B cells (Figure 1). Thus, although the presence of IgM-RF showed a significant association with disease activity in children with polyarticular JRA, it did not correlate with levels of CD5+ B cells.

DISCUSSION

This study was conducted to determine whether children with JRA have elevated levels of CD5+ B cells, and to determine whether these levels correlate with either disease activity or the presence of IgM-RF detected by ELISA. Our data show that, in comparison with normal controls and children with other collagen vascular diseases, expanded populations of these cells are demonstrable in the circulation of patients with polyarticular JRA, but not those with pauciarticular JRA.

Our control population was slightly younger than the group with polyarticular JRA, but the differences in age among the 4 groups studied were not statistically significant. CD5+ B cell levels are high at birth (90–100% of B cells in cord blood are CD5+ [10]) and tend to fall with age; thus, the slightly younger age of the healthy controls would, if anything, obscure, rather than artificially create, significant differences between their levels of CD5+ B cells and those in children with polyarticular disease. Although CD5+ B cell levels were also slightly increased in children with pauciarticular JRA compared with the healthy controls and the children with other collagen vascular diseases (28%, versus 20% in children with collagen vascular diseases and 22% in the controls), the differences were not significant.

We have also shown that increases in circulating levels of CD5+ B cells are strongly associated with active disease in polyarticular JRA. Increased levels in this patient group occurred only among children with active disease. Although Martini et al demonstrated

that levels of circulating CD5+ B cells commonly change in children with JRA irrespective of disease activity (6), their patients were not divided by type of disease onset. Thus, the inclusion of patients with pauciarticular disease in an analysis of the relationship between CD5+ B cell levels and disease activity in JRA might have obscured the relationship demonstrated here, since these levels do not appear to vary with disease activity in pauciarticular disease as they do in polyarticular disease.

A similar association between increased levels of CD5+ B cells and disease activity has not been reported in adult RA patients. Although individual adult patients with systemic lupus erythematosus and RA have been shown to have decreased levels of CD5+ B cells during periods of inactive disease (11), evidence suggests that CD5+ B cell levels in adults are determined primarily by genetic factors (12) and are stable both in healthy adults and in patients with RA (13).

We have also confirmed the findings of Moore et al (1) that IgM-RF is associated with disease activity in polyarticular JRA. As we predicted, higher levels of CD5+ B cells tended to be associated with the presence of IgM-RF as determined by ELISA, although this correlation did not reach statistical significance. Although it is possible that the presence of IgM-RF and the expansion of the CD5+ B cell population may be independent events, the occurrence of elevated CD5+ B cell levels in diseases associated with IgM-RF, but not in other autoimmune diseases (5), suggests that the two are related.

The role of CD5+ B cells in JRA is probably linked to the capacity of these cells to produce IgM-RF (3,4). These, in turn, may play a direct role in the pathophysiology of this and other autoimmune diseases. Evidence suggests that IgM-RF alters complement-mediated immune complex processing. IgM-RF from adults impedes complement-mediated inhibition of immune precipitation (14), prevents complement binding to immune complexes, and prevents the binding of those complexes to complement receptors on erythrocytes, while at the same time allowing activation of the classical pathway to proceed (15). In addition, recent work in our laboratory indicates that sera from children with polyarticular JRA have reduced capacity to prevent precipitation of immune complexes, and that this is due, in part, to the presence of IgM-RF (Jarvis JN et al: unpublished data).

Thus, it seems reasonable to postulate that the expansion of the population of CD5+ B cells is responsible for the presence of IgM-RF in polyarticular JRA and adult RA. Whether this expansion represents dysregulation of a separate cell population normally

kept within strict limits, or is a sign of specific or nonspecific B cell activation, remains unknown, but we believe answers to these questions will provide important insights into the pathophysiology of rheumatic diseases in which rheumatoid factor plays a role.

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