The Objective of this cross-sectional study is to investigate serum and synovial fluid type 1 (Th1) and type 2 (Th2) T cell activities in children with juvenile rheumatoid arthritis [JRA], and to correlate Th1/Th2 cell activities with severity of the disease. Among attendants of Pediatric Rheumatology Outpatient Clinic Tanta University Hospital, 27 children with JRA, 15 polyarticular & 12 pauciarticular, were enrolled in the current study. Serum and synovial fluid samples were collected from all patients and from 10 children with congenital orthopedic deformities during corrective orthopedic surgery. Those 10 children constitute the control group. Peripheral blood mononuclear cells were isolated from patients and controls. Primary T cell activity in these mononuclear cells was enhanced by means of anti-CD3/anti-CD28, which mimics stimulation of T cells by activation of the T cell receptor and a major co-stimulatory signal. Interferone gamma (IFNγ) and interleukin 4 (IL4) production were quantified as measures of Th1 and Th2 cell activity respectively. ESR, C-RP, and grading of clinical activity were determined as parameters of disease activity and correlated with serum and synovial fluid Th1/Th2 cell activity. Results: (1) Peripheral T cells from JRA patients produced significantly less IFNγ [77±35 pg/ml vs 128±30 pg/ml, P<0.05] and more IL4 [151±28 pg/ml vs 97±25 pg/ml, P<0.05] than T cells from matched controls. (2) Synovial fluid from JRA patients showed significantly more IFNγ [189±51 pg/ml vs 108±47 pg/ml, P<0.05] and less IL4 [54±14 pg/ml vs 94±35 pg/ml, P<0.05] than T cells from matched controls. (3) Peripheral blood and synovial fluid IFNγ and IL4 activities correlated significantly with clinical and laboratory indices of disease activity. In conclusions: (1) The balance between Th1/Th2 cells activity seems crucial in controlling the severity of JRA in children. (2) Skewing towards Th1 cell predominance is suggested to be the pathogenic mechanism underlying JRA activity. (3) Redressing Th1/Th2 balance -- immune deviation -- could be suggested as the future therapy in JRA. This could be attained by the use of different lines of biotechnological therapeutic advances including inhibitors of proinflammatory cytokines that could change Th1/Th2 cell balance in favor of Th2 cell activity and/or by prevention of the migration of Th1 cells using IL-4, IL-10, CD4 monoclonal antibodies, anti-ICAM-1 antibodies, or combination therapy.
INTRODUCTION

Juvenile rheumatoid arthritis (JRA) is a chronic inflammatory disease of unknown etiology, characterized by destruction of joints (Cassidy and Petty, 1990; Fox, 1997; Simmons et al., 1996). The presence of CD4+ T-helper cells in the synovium of adults (Jarvis, 1998; Panayi et al., 1992) and children (Murray et al., 1996) with rheumatoid arthritis has led to the generally accepted hypothesis that aberrant activation or regulation of acquired immunity is central to the pathogenesis of this family of diseases. However, this hypothesis remains unproven, and, indeed, a specific role for T cells in the process of chronic synovitis in rheumatoid diseases has yet to be identified for either adults or children (Jarvis, 1998; Murray et al., 1996).

The concept of T helper cells heterogeneity with Th1 and Th2 subclasses, distinguished on the basis of their cytokines repertoire, has been a useful paradigm in immunological studies of many autoimmune diseases (Mosmann & Sad, 1996; Romagnani, 1995; Swain, 1994). Th1 cells are involved in cellular immunity and interferon-gamma [IFN-γ] is the prototype Th1 cytokine (Thorpe et al., 1992). On the other hand, Th2 cells are involved in humoral immune response and interleukin 4 [IL-4] is the prototype Th2 cytokine (Van der Pouw-Karan et al., 1992).

Several studies, in adults (Dolhain et al., 1996; Lepore, 1994; Madson et al., 1994) and children (Mongge et al., 1995; Ozen et al., 1998; Rooney et al., 1995; Woo, 1993), had tried to correlate clinical parameters of rheumatoid arthritis and the spontaneous production of IFN-γ and IL-4 by T-cells from synovial fluid and/or peripheral blood. Although the presence of Th2 cells activity in RA has been shown by means of in situ production of IL-4 (Lepore, 1994; Woo, 1993), numerous studies have shown an intra-articular predominance of Th1 cell activity by means of in situ production of IFN-γ (Dolhain et al., 1996; Mongge et al., 1995; Ozen et al., 1998; Rooney et al., 1995; Woo, 1993). Nevertheless, the association of these T cells activities with inflammation and joint damage in RA remains obscure. Again, these studies have revealed undetectable or low levels of IFN-γ and IL-4 in peripheral blood. Both findings make it difficult to visualize the role of cytokines in disease activity (Dolhain et al., 1996; Rooney et al., 1995; Woo, 1993).

The objective of the present study was to determine the pattern of expression of Th1/Th2 cell cytokines both in synovial fluid and peripheral blood in children with juvenile rheumatoid arthritis and to correlate these cytokines with clinical and laboratory parameters of activity of the disease.

PATIENTS AND METHODS

Patients:

Among attendants of Pediatric Rheumatology Outpatient Clinic, Tanta University Hospital, 27 children with JRA were included in the current study. Patients were enrolled only if they fulfilled European League Against Rheumatism [EULAR ] diagnostic criteria of JRA (Brewer & Bass Baum, 1977). They included 12 boys and 15 girls, aged 2.3-15.2 yrs (mean 10.2 yr). The duration of JRA ranged from 3-210 mo (mean, 51.8 mo). The onset of JRA was systemic in 7 patients (all polyarticular), polyarticular in 8 patients (all rheumatoid factor -ve), and pauciarticular in 12 patients
- Synovial fluid samples were examined for IFN-γ and IL-4 levels.

[ II ] Peripheral Blood Mononuclear Cell Cultures (June et al., 1994; Van Lier et al., 1988).
- Peripheral blood was diluted 1:1 with Dulbecco's modified Eagle's medium (DMEM, Gibco 074-01600; 24 mM NaHCO₃) supplemented with glutamine (2 mM), penicillin (100 U/ml), and streptomycin sulphate (100 mg/ml; DMEM⁺) and MNC were isolated by density centrifugation using Ficoll-Pague (Pharmacia). Viability of cells, checked by trypan blue exclusion, was always > 95%. Subsequently, MNC were cultured for 48 hours in DMEM⁺ supplemented with 10% human male AB⁺ serum.
- Spontaneous production of IFN-γ and IL-4 by these cells was increased by anti-CD3 and anti-CD28 antibodies (1:1000 v/v, CLB, Amsterdam, the Netherlands). This stimulus activates T cells through the CD3 complex together with a costimulatory signal via the CD28 molecule. After 48 hours of culture, conditioned media were harvested and freed of cellular material by centrifugation, frozen in liquid nitrogen, and stored at -70°C.

[ III ] IFN-γ (Thorpe et al., 1992) and IL-4 (Van der Pouw-Karan et al., 1992)
Measurement:
IFN-γ and IL-4 were assessed both in synovial fluid and in peripheral blood MNC cultures by ELISA (Medgenix, Flerus, Belgium) according to the manufacturer's guidelines. Detection limits were 50 pg/ml for IFN-γ, and 16 pg/ml for IL-4.

Data were expressed as means ± SD. Student t test was used to compare between total children with JRA and total controls. The correlation studies were done by Pearson’s correlation coefficient.

RESULTS

Results of the present study are illustrated in tables 1&2 and figures 1&2 and can be summarized as follows:

[ A ] SYNOVIAL FLUID CYTOKINES:

a. IFN-γ and IL-4 production in synovial fluid: [Table, 1]
- IFN-γ production in synovial fluid from Children with JRA was significantly higher than that in matched controls (P<0.05).
- IL-4 production in synovial fluid from Children with JRA was significantly lower than that in matched controls (P<0.05).
- IFN-γ/IL-4 ratio in synovial fluid from Children with JRA was significantly higher than that in matched controls (P<0.05).

b. Relation of synovial fluid IFN-γ and IL-4 to disease activity: [Table, 2]
- IFN-γ production in synovial fluid from Children with JRA correlated positively in a significant manner with clinical scores of activity.
as well as with an increase of ESR(P<0.05) and C-RP(P<0.001).

- IL-4 production in synovial fluid from Children with JRA correlated negatively in a significant manner with clinical scores of activity (P<0.001) as well as with an increase of ESR(P<0.05) and C-RP(P<0.001).

c. **Relation of synovial fluid IFN-γ/IL-4 ratio to disease activity:** [Table, 2]

- IFN-γ/IL-4 ratio in synovial fluid from Children with JRA correlated positively in a significant manner with clinical scores of activity (P<0.001) as well as with an increase of ESR(P<0.05) and C-RP(P<0.001).

[B] **PERIPHERAL BLOOD T CELL CYTOKINES:**

a. **IFN-γ and IL-4 production from peripheral blood T cells:** [Table, 1]

- IFN-γ production of peripheral blood T cells from Children with JRA was significantly lower than that in matched controls (P<0.05).

- IL-4 production of peripheral blood T cells from Children with JRA was significantly higher than that in matched controls (P<0.05).

- IFN-γ/IL-4 ratio in peripheral blood T cells from Children with JRA was significantly lower than that in matched controls (P<0.05).

b. **Relation of IFN-γ and IL-4 production from peripheral blood T cells to disease activity:** [Table, 2]

- IFN-γ production in peripheral blood T cells from Children with JRA correlated negatively in a significant manner with clinical scores of activity (P<0.001) as well as with an increase of ESR(P<0.05) and C-RP(P<0.001).

- IL-4 production in peripheral blood T cells from Children with JRA correlated positively in a significant manner with clinical scores of activity (P<0.001) as well as with an increase of ESR(P<0.05) and C-RP(P<0.001).

c. **Relation of IFN-γ/IL-4 ratio from peripheral blood T cells to disease activity:** [Table, 2]

- IFN-γ/IL-4 ratio in peripheral blood T cells from Children with JRA correlated negatively in a significant manner with clinical scores of activity (P<0.001) as well as with an increase of ESR(P<0.05) and C-RP(P<0.001).

[C] **RELATION OF IFN-γ AND IL-4 PRODUCTION IN SYNOVIAL FLUID TO IFN-γ AND IL-4 PRODUCTION BY PERIPHERAL BLOOD T CELLS:** [Figures 1&2]

- IFN-γ production of peripheral blood T cells from Children with JRA correlated negatively in a significant manner with IFN-γ production in synovial fluid in children with JRA [r = -0.79, P<0.001].

- IL-4 production of peripheral blood T cells from Children with JRA correlated negatively in a significant manner with IL-4 production in synovial fluid in children with JRA [r = -0.64, P<0.001].
Table 1] Synovial Fluid and Peripheral Blood IFN-γ and IL-4 in Children with JRA vs Controls

<table>
<thead>
<tr>
<th></th>
<th>Children with JRA</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synovial Fluid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFN-γ (pg.ml)</td>
<td>189±51*</td>
<td>108±47</td>
</tr>
<tr>
<td>IL-4 (pg.ml)</td>
<td>54±14*</td>
<td>94±35</td>
</tr>
<tr>
<td>IFN/IL-4 ratio</td>
<td>3.5±1.4*</td>
<td>1.1±0.4</td>
</tr>
<tr>
<td>Peripheral Blood</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFN-γ (pg.ml)</td>
<td>77±35*</td>
<td>128±30</td>
</tr>
<tr>
<td>IL-4 (pg.ml)</td>
<td>151±28*</td>
<td>97±25</td>
</tr>
<tr>
<td>IFN/IL-4 ratio</td>
<td>0.5±0.4*</td>
<td>1.3±0.3</td>
</tr>
</tbody>
</table>

*Significantly different from control values ( P<0.05 ).

Table [2] Correlations Between IL-4, IFN-γ, And IFN-γ / IL-4 Ratio In Children With JRA And Clinical & Laboratory Indices Of JRA Activity

<table>
<thead>
<tr>
<th>Indices Of JRA Activity</th>
<th>IFN-γ Synovial Fluid</th>
<th>IFN-γ Peripheral Blood</th>
<th>IL-4 Synovial Fluid</th>
<th>IL-4 Peripheral Blood</th>
<th>IFN-γ / IL-4 Ratio Synovial Fluid</th>
<th>IFN-γ / IL-4 Ratio Peripheral Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P</td>
<td>r</td>
<td>P</td>
<td>r</td>
<td>P</td>
</tr>
<tr>
<td>ESR</td>
<td>+0.67</td>
<td>&lt;0.05</td>
<td>-0.67</td>
<td>&lt;0.05</td>
<td>-0.67</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>C-RP</td>
<td>+0.87</td>
<td>&lt;0.001</td>
<td>-0.87</td>
<td>&lt;0.001</td>
<td>-0.87</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Clinical Scores</td>
<td>+0.89</td>
<td>&lt;0.001</td>
<td>-0.89</td>
<td>&lt;0.001</td>
<td>-0.89</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Figure [1] Correlation between synovial fluid and peripheral blood IFN-γ in children with JRA

Figure [2] Correlation between synovial fluid and peripheral blood IL-4 in children with JRA
DISCUSSION

Most data concerning the role of CD4+ T-helper cells cytokines in rheumatoid arthritis have been obtained in adult RA (Dolhain et al., 1996; Lepore, 1994; Madson et al., 1994; Schultz-Koops, 1995). To the best of the available knowledge, the current study is the first study concerned with investigating peripheral blood as well as synovial fluid T-helper cell cytokines in children with JRA. Previous studies in children with JRA were concerned with peripheral blood cytokines and failed to visualize the role of cytokines in disease activity (Mongge et al., 1995; Ozen et al., 1998; Rooney et al., 1995; Woo, 1993).

In the current study, it is shown that in children with JRA, the cytokine pattern in synovial fluid shows relative predominance of IFN-γ over IL-4 compared with that found for controls [table 1]. This shows that the potency of synovial fluid Th1 over Th2 cell activity is higher in children with JRA vs controls.

On the contrary, the cytokine pattern of blood T cells, in the current study, shows relative predominance of IL-4 over IFN-γ compared with that found for controls [table 1]. This shows that the potency of peripheral blood Th1 over Th2 cell activity is lower in children with JRA vs controls.

Therefore, it could be speculated that, in children with JRA, Th1/Th2 imbalance is strongly suggested, with intra-articular Th1 predominance and systemic Th2 predominance.

Importantly, this study shows positive correlation between in vivo determined synovial fluid Th1 cell activities and disease parameters of JRA as well as an inverse correlation of these parameters of disease with Th2 cell activity, most clearly expressed by an increase in Th1/Th2 cell cytokine ratio. Again, this study shows an inverse correlation between ex vivo determined peripheral Th1 cell activities and disease parameters of JRA as well as a positive correlation of these parameters of disease with Th2 cell activity, most clearly expressed by a decrease in Th1/Th2 cell cytokine ratio [table 2].

These data pinpoint to a pivotal pathogenic role of Th1 cell cytokines and a protective role of Th2 cell cytokines in JRA. The current data also supports previous concepts that Th1 cells are the villains and Th2 cells are the white knights during chronic arthritis (van Room et al., 1997; van Room et al., 1995).

These findings were surprising, as it was expected that predominant intra-articular Th1 cell activity, leading to activation of macrophages and subsequently inflammation, would be found in the periphery as well. Although surprising, our finding corroborate previous reports on a lower IFN-γ production by mononuclear cells from the peripheral blood of children with JRA vs controls stimulated with anti-CD3 (Mongge et al., 1995; Ozen et al., 1998; Raziuddin et al., 1998). Also, our finding corroborates recently reported data in adults that suggested Th1 cell preponderance in RA synovium (Aarvak et al., 1999; De Benedetti et al., 1997; Jarvis, 1998; Jarvis et al., 1997; Raziuddin et al., 1998).

These findings may be explained by a selective migration of Th1 cell from the peripheral blood into the inflamed joint and consequently a decrease in IFN-γ
producing cell in the peripheral blood. Recently, selective migration of Th1 cells compared with Th2 cells into inflamed joints has been shown in mice (Austrup et al., 1997). Furthermore, selective migration of Th1 cells has been suppressed after treatment of JRA patients with antibodies against ICAM-1 which prevents transendothelial migration of T cells in inflammatory sites (Schultz-Koops, 1995). The significant negative correlations detected in the current study between synovial fluid and peripheral blood cytokines [figures 1&2] supports this hypothesis.

Prevention of the migration of T cells resulted in a specific increase in the number of IFN-γ-producing cells in peripheral blood from these patients, whereas no increase IL-4-producing cells was seen. The increase in IFN-γ-producing cells in peripheral blood was related to clinical improvement (Schultz-Koops, 1995).

Low peripheral Th1 cell activity and high Th2 cell activity might also be the consequence of factor, such as IL-10, and TGF-β, which can change Th1/Th2 cell balance in favor of Th2 cell activity (Cush et al., 1995; Kutsikis et al., 1994; Lotz et al., 1990; Miossec et al., 1990). In JRA, these factors have been shown to be produced by the intra-articular activated macrophages (Cush et al., 1995; Miossec et al., 1990; van Room et al., 1995; van Room et al., 1996). These mediators might occur in the periphery (Cush et al., 1995). However, intra-articular Th1 cell activating signals including IL-1 and TNF-α are produced in a significant manner in RA and may overcome these suppressive signals, maintaining a predominance of Th1 cell activity (Cope and Maini, 1995; De Benedetti et al., 1997; Muller et al., 1998). On the other hand, in the absence of Th1 cell activating signals in the periphery, these suppressive factors may lead to a change in the T cell balance in favor of Th1 over Th2 cell activity (Miossec et al., 1990).

These data shows that Th1/Th2 cell cytokine imbalance is strongly associated with the severity of JRA. Consequently, redressing Th1/Th2 balance – immune deviation – could be suggested as the future therapy in JRA. This could be attained by the use of different lines of biotechnological therapeutic advances (Cush & Kavanaugh, 1995; Elliott & Maini, 1994; Fox, 1995; Kavanaugh & Lipsky, 1994) including inhibitors of IL-1 and TNF-α (Arned & Dayer, 1995; Duncan, 1998; Firestein & Zvaifler, 1997; Kavanaugh et al, 1998; Koopman & Moreland, 1998) that could change Th1/Th2 cell balance in favor of Th2 cell activity and/or by prevention of the migration of Th1 cells using IL-4, IL-10 (Joosten et al., 1997), CD4 monoclonal antibodies (Rep et al., 1997), combination therapy with a TNF antagonists and a CD4 monoclonal antibody (Morgan et al., 1997; Williams et al., 1994), or anti-ICAM-1 antibodies (Schultz-Koops, 1995).

However, while these novel therapeutic lines yields remarkable encouraging results in experimental and adult RA, the ultimate utility of these agents in pediatrics will only be delineated after long-term researches and clinical observations in pediatric rheumatology clinics. The introduction of these novel therapeutic lines could herald a new era in pediatric rheumatology therapeutics. Having been disappointed by so many biologic agents in the past, we wish that we will be finally having a therapy that can provide considerable tangible benefit for our JRA patients.

**CONCLUSIONS**

(1) The balance between Th1/Th2 cells activity seems crucial in controlling the severity of JRA in children.
(2) Skewing towards Th1 cell predominance is suggested to be the pathogenic mechanism underlying JRA activity.

(3) Redressing Th1/Th2 balance – immune deviation – could be suggested as the future therapy in JRA. This could be attained by the use of different lines of biotechnological therapeutic advances including inhibitors of IL-1 and TNF-α that could change Th1/Th2 cell balance in favor of Th2 cell activity and/or by prevention of the migration of Th1 cells using IL-4, IL-10, CD4 monoclonal antibodies, combination therapy with a TNF antagonists and a CD4 monoclonal antibody, or anti-ICAM-1 antibodies.

REFERENCES


