Purified Protein Derivative Response in Juvenile Idiopathic Arthritis

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ABSTRACT. *Objective.* To examine the purified protein derivative (PPD) response that develops depending upon Th1 immune response in children with juvenile idiopathic arthritis (JIA).

Methods. PPD skin test was performed in 115 children with JIA who were vaccinated with bacillus Calmette-Guerin (BCG), and then they were compared to the PPD response of 45 healthy children of the same age who were vaccinated with BCG. Children with a PPD induration ≥ 5 mm were accepted as PPD-positive. PPD induration ≥ 10 mm was accepted as a limit for suspecting tuberculosis.

Results. PPD induration size and PPD positivity rates (PPD ≥ 5 mm) of children with JIA were significantly lower than those of healthy children. The mean of PPD induration size was significantly lower ($p < 0.0001$) in patients with either 1 BCG vaccine (3.7 ± 3.6) or more than 1 BCG vaccine than controls with either 1 BCG vaccine (7.10 ± 3.2) or more than 1 BCG vaccine (10.05 ± 4.1). PPD was positive in 35.9% of patients with JIA vaccinated once (n = 32), in 50% of patients with JIA vaccinated more than once (n = 13), in 82.1% of controls vaccinated once (n = 23), and in 88.2% of controls vaccinated more than once. This result was statistically significant (patients, $p = 0.03$; controls, $p = 0.039$). It was determined that neither the activity of the disease nor the use of corticosteroid and methotrexate affected the PPD response.

Conclusion. The response to PPD, which is one of the Th1 cell-type responses, was significantly lower in BCG-vaccinated children with JIA compared to healthy children. (J Rheumatol First Release Aug 1 2009; doi:10.3899/jrheum.090173)

Key Indexing Terms:
JUVENILE IDIOPATHIC ARTHRITIS   PURIFIED PROTEIN DERIVATIVE TEST
                         TUBERCULIN SKIN TEST

Juvenile idiopathic arthritis (JIA) is a disease that is commonly seen along with peripheral arthritis, and its pathogenesis is not completely known. Infections, autoimmunity, trauma, stress, and genetic tendency have been proposed as the predisposing factors. The CD4 T lymphocytes [T helper (Th) cells], a subgroup of T lymphocytes, have an important role in its immunopathogenesis[1-4]. Th have 2 subgroups: Th1 and Th2. Any imbalance of those cells is thought to cause several different groups of diseases such as atopia, allergic diseases, and autoimmune diseases. It is assumed that Th1 type cell response in autoimmune inflammatory diseases and Th2 type cell response in atopical diseases are dominant[4-7].

Purified protein derivative (PPD, tuberculin) skin reaction is a late hypersensitivity reaction that develops classically depending upon Th1 type cytokine response. It shows whether a person has been previously exposed to *M. tuberculosis*, but it does not inform about the disease[8,9]. PPD response is a widely used indicator of tuberculosis (TB). Children with JIA whose Th1 cell response is presumably dominant are expected to show higher PPD response in comparison to healthy children. Although there are observations based on *in vitro* and *in vivo* studies on the suppression of delayed hypersensivity response in adults with rheumatoid arthritis (RA)[5,9-12], there are no reports in the literature of similar studies in children with JIA. Therefore, we assessed the reaction to PPD test in JIA with assumed dominance of the Th1 cell immune response.

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MATERIALS AND METHODS

Study groups. One hundred fifteen children (70 girls, 45 boys) diagnosed with JIA according to International League of Associations for Rheumatology criteria[13] without any chronic or acute infection were included in the study, in addition to 45 healthy children (22 girls, 23 boys) matched for age and sex. The type and activity of the illness, the number of joints involved, presence of uveitis, HLA-B27, antinuclear antibodies (ANA) and rheumatoid factor (RF), drug usage, and drug amounts were recorded. The families were questioned about when and how many times

the children were vaccinated and if there was any history of TB in the family. Informed consents were taken from the families of both patients and healthy children.

This study was approved by the ethical committee of Cerrahpasa Medical School.

PPD hypersensitivity test (tuberculin skin test). Tuberculin skin test was performed for both patients and healthy children by the same investigator, and the induration diameter was observed 48 h later by the same investigator. An induration ≥ 5 mm was accepted as a positive result. An induration ≥ 10 mm was accepted as the limit value for TB disease. For the latter patients, TB was further tested by a detailed history, sedimentation rate, and chest radiography. During the period of PPD testing all children received their previous therapy without any change.

Statistical analysis. The data were analyzed by SPSS 11.0. Chi-squared, Student t-test, and Mann-Whitney U-tests were used for the comparison of data, and Pearson correlation test was used for the calculation of correlation. A p value < 0.05 was accepted as statistically significant.

RESULTS

There was no statistical difference (p > 0.05) between the mean age of children with disease (8.27 ± 4.34 yrs, range 1.25–18.58) and healthy children (8.11 ± 3.27 yrs, range 2.83–16). In the JIA group of 115 children, the subtype of the disease was systemic in 26, oligoarticular in 41 (persistent oligoarthritis in 40 and extended oligoarthritis in 1 patient), enthesitis-related arthritis in 15, polyarticular in 29, and juvenile psoriatic arthritis in 4. At the beginning of our study, 32 (27.8%) of the JIA group were in an active stage of the disease according to the criteria[14,15]. The mean (± standard deviation, SD) number of joints involved was 3.63 ± 2.079 (range 0–10) and the mean duration of the disease was 3.89 ± 2.85 years. Six patients with JIA (4.3%) had uveitis, 12 (10.4%) were HLA-B27-positive, 5 (4.3%) were RF-positive, and 34 (29.6%) were ANA-positive. During the study 55 patients used 5 ± 7.38 (range 2.5–40) mg/day corticosteroid, 73 used 10 ± 4.4 (3–20) mg/week methotrexate, and 17 used 800 ± 758 (5–2000) mg/day sulfasalazine.

Evaluation of PPD results. The mean PPD induration size in patients with JIA (4.12 ± 5.24 mm, range 0–20 mm) independent of bacillus Calmette-Guerin (BCG) vaccine count, was significantly lower than that in controls (7.83 ± 3.47 mm, range 0–16 mm) (p < 0.0001).

Forty-five patients with JIA (39.1%) and 38 controls (84.4%) were PPD-positive (p = 0.03). Twenty-four patients (21%) and 19 controls (42.2%) had a PPD induration ≥ 10 mm (p = 0.03).

PPD reaction was not observed in 28 patients (24.34%) and 3 healthy children (6.6%) (p = 0.013).

Relationship of BCG vaccine and PPD induration. The duration following the last BCG vaccine was not different between patient (6.87 ± 3.82 yrs) and control (6.09 ± 3.36 yrs) groups (p = 0.11).

Of the patients, 77.4% had been vaccinated only once, and of the controls 62.2% were vaccinated only once (p > 0.05). The number of BCG vaccinations had no effect on controls or on patients with JIA, indicating that the differ-

ence in PPD induration size was independent of BCG count (Table 1).

The durations after the latest BCG and vaccinating counts were not different between PPD-positive and PPD-negative patients with JIA (p = 0.21, p = 0.19, respectively).

Also, BCG vaccine count or duration after the latest BCG was not different between the patients with ≥ 10 mm PPD induration and ≤ 10 mm PPD induration (p = 0.20 and p = 0.18, respectively). No active TB was defined in those cases.

Effect of medication on PPD response. The PPD induration size for corticosteroid users (n = 55) was not different from nonusers (3.86 ± 5.36 mm and 4.65 ± 5.05 mm, respectively). Further, the PPD dimension was not different between methotrexate users (n = 73) and nonusers (4.33 ± 5.54 mm and 3.89 ± 4.91 mm, respectively). There was no difference by means of PPD positivity between both corticosteroid (34%, 31%) or methotrexate (29%, 32%) users or nonusers. Seventeen patients took sulfasalazine. Statistical analysis could not be performed since the number of patients was not large enough. Anti-tumor necrosis factor (TNF) drugs were not used for any of the patients.

Effect of activity, type, and duration of the disease. The PPD positivity ratio, PPD induration diameter, and presence of an induration ≥ 10 mm was not different in those patients in the active phase of the disease in comparison to those without an active disease (p = 0.82; Table 2). Hence, disease activity was independent from the effect on the response to the PPD test. In the patients with active disease, a correlation between the duration of the active disease and the PPD induration could not be demonstrated. The positivity of the PPD response in those with active disease (n = 12, 37.5%) and without active disease (n = 33, 39.7%) was significantly lower (p = 0.029 and p = 0.031, respectively) compared to that in the healthy control group. The incidence of a PPD induration ≥ 10 mm among those with active disease (n = 7, 21.8%) and inactive disease (n = 17, 20.4%) was also significantly lower (p = 0.031, p = 0.032, respectively) compared to the healthy controls (Table 2).

Effect of familial TB history to PPD response. There were 19 (16.5%) patients with a TB history in the family. The mean PPD indurations, PPD positivity rates, and ≥ 10 mm PPD induration rates were not different for patients with or without a family history of TB (p = 0.238, p = 0.057, and p = 0.2, respectively). No TB case was reported for the families of healthy children.

DISCUSSION

We studied PPD skin response in patients with JIA. Both JIA as a disease process and PPD as a skin reaction are Th1 type lymphocyte-dependent processes. To our knowledge, this study is the first to investigate the PPD response in patients with JIA. The results are noteworthy in that, first, compared to an age- and sex-matched group of healthy chil-

Table 1. PPD response according to BCG vaccine status (no. of BCG vaccinations).

| | Children with JIA, n = 115 | | Healthy Children, n = 45 | | p | | | |
	BCG = 1, n = 89	BCG > 1, n = 26	BCG = 1, n = 28	BCG > 1, n = 17	p1	p2	p3	p4
Age, yrs, mean ± SD	7.56 ± 3.9	9.73 ± 5.5	7.3 ± 2.9	8.4 ± 4.1	NS	NS	NS	NS
PPD induration, mm, mean ± SD	3.7 ± 3.6	5.76 ± 4.4	7.1 ± 3.2	10.05 ± 4.1	NS	NS	< 0.001	< 0.001
PPD induration								
≥ 5 mm, n (%)	32 (35.9)	13 (50)	23 (82.1)	15 (88.2)	NS	NS	0.03	0.039
≥ 10 mm, n (%)	17 (19.1)	7 (26.9)	8 (28.5)	11 (64.7)	NS	NS	0.046	0.029

NS: statistically not significant (p > 0.05). p1: between JIA patients vaccinated ≥ 1 time with BCG. p2: between healthy children vaccinated ≥ 1 time with BCG. p3: between JIA patients and healthy children who were vaccinated once. p4: between JIA patients and healthy children who were vaccinated > 1 time. BCG: Bacillus Calmette-Guerin; PPD: purified protein derivative; JIA: juvenile idiopathic arthritis.

Table 2. PPD response of patients with JIA according to disease activity.

| | JIA, n = 115 | | Controls, n = 45 | p | | |
	Active Disease	Inactive Disease		p1	p2	p3
PPD induration, mm, mean ± SD	4 ± 5.66	4.17 ± 5.10	7.83 ± 3.47	NS	< 0.001	< 0.001
PPD induration						
0–4 mm, n (%)	20 (62.5)	50 (60.2)	7 (15.5)	NS	0.016	0.015
≥ 5 mm, n (%)	12 (37.5)	33 (39.7)	38 (84.4)	NS	0.031	0.029
≥ 10 mm, n (%)	7 (21.8)	17 (20.4)	20 (44.5)	NS	0.033	0.032

NS: statistically not significant (p > 0.05). p1: between patients with active and inactive disease. p2: between active disease and healthy children. p3: between inactive disease and healthy children.

dren, both the PPD positivity ratio and the PPD induration diameter were found to be significantly lower in children with JIA whether vaccinated once or more than once with BCG. Second, approximately one-quarter (24.3%) of the JIA group, in comparison to 6% of the healthy controls, showed no response to the PPD test. Third, the disease activity in the JIA patients did not affect the response to the PPD test. Finally, a statistically significant difference in the PPD positivity ratio, the mean PPD induration diameter, and the detection of an induration diameter ≥ 10 mm among those JIA patients with and without a family history of TB was not demonstrable. All these results, expected to have been raised in the patients with JIA, were on the contrary significantly decreased. In our country the BCG vaccination is given according to the recommendation of the Turkish Ministry of Health. The first dose is given during the second or third postnatal month and the second dose at 6 or 7 years of age if there is a negative PPD result[8].

It has been shown that the response to the PPD test can change with the number of BCG vaccinations[16], the duration following the last vaccination[17], the environmental presence of mycobacterium other than *M. tuberculosis*[18], and use of corticosteroids[19] or of immunosuppressive drugs. In our study, the number of vaccinations and the duration following the last vaccination in the JIA and the control groups were comparable. Further, it was determined that

PPD induration size or PPD positivity rates were not affected for patients who have used methotrexate and low-dose corticosteroid (5 mg/day). No statistical difference was reported in a previous study between patients with RA who were given immunosuppressive treatment or not by means of PPD induration and PPD positivity[20]. No such comparison could be performed in our study, since the number of our cases was not enough to perform a statistical analysis and we had no case that was given an anti-TNF treatment.

The reason for the suppression observed in the response to the PPD skin test in the patients with JIA is not understood, although some reasons can be hypothesized. First, there may be an imbalance in the response of the Th1 type of cell known to release cytokines like interferon-γ[21]. Accordingly, in JIA the decreased response to the PPD test may result from the suppression of the release of this cytokine or from the accumulation of the Th1 cells at the sites of the lesions. This may also result from an immunosuppressive effect of the drugs used. Previously, it was shown that low-dose corticosteroid usage for less than 15 days did not affect the PPD response[19]. Our study also demonstrates that longterm low-dose corticosteroid usage did not affect the PPD response. However, we cannot explain our observation of the ineffectiveness of the disease activity response to the PPD test. We also cannot account for the PPD response of our patients at the stage of diagnosis, as

none of them were newly diagnosed, which may be an important lack of data in our study.

The observed suppression of the Th1 type of cell response in our group of patients with JIA suggests the vulnerability of patients with systemic lupus erythematosus and RA[22,23]. This may be of concern especially for those countries with a high prevalence of TB. Our results show that the PPD response that has been used for years in the screening for TB will not be accurately informative in cases of JIA; those cases therefore would require the application of more sensitive tests[24].

The observation of a significant suppression in the Th1-dependent response to the PPD test in JIA appears to conflict with the immunopathology of JIA assumed to be characterized by predominance of Th1 type of cell activity. Further investigation of this in children with JIA may shed light on the immunopathogenesis of the disease.

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