Intradermal Tests in SLE versus in Other Connective Tissue Diseases

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Negative intradermal tests with PPD were found in 88% of SLE cases, 52% of cases with other connective tissue diseases and allied conditions and 6% of normal controls. Negative SK-SD tests were in 86% of SLE, 42% of others and 40% of controls. Negative Candida tests were in 36% of SLE, 16% of others and 0% of controls. Positive DNA tests were 40% of SLE, 13% of others and 0% of controls. SLE cases with positive for DNA test appeared clinically active and to have lymphopenia in the peripheral blood. Lymphopenia lower than 1,500/mm³ was frequently seen in SLE (88%) than in others (42%). There was no preponderance of negative result of intradermal tests in the aged.

Key Words: PPD, SK-SD, Candida, DNA, Age lymphopenia, Leukocyte migration inhibition test, Disease activity.

Through recent advances of immunology, various methods have been explored for the detection of immunologic abnormalities in various disorders. Intradermal tests are well known as one of the in vivo tests to evaluate the impaired cellular immunity. In this report, we present a result of intradermal tests in patients with SLE and other connective tissue diseases and allied conditions using 4 different antigens available for clinical use. Age, peripheral leukocyte migration inhibition test with DNA and disease activity were included for the analysis of intradermal tests.

MATERIALS AND METHODS

Sixteen healthy subjects, 8 male and 8 female with an age range of 18 to 48, were included as a normal control group.

Sixteen patients with SLE, 2 male and 14 female with an age range of 18 to 65, were investigated. Among 16 cases, 8 patients were without treatment and other 8 patients were with treatments of glucocorticoids or immunosuppressants such as cyclophosphamide and azathiopurine, or a combination of them. As to the disease activity of SLE, cases were regarded as clinically active when they had more than one item as follows: fever higher than 38°C, skin eruptions or oral ulcers, polyarthritis, cytopenia of peripheral blood and active nephropathy.

Thirty one cases with connective tissue disease and allied conditions of immunological abnormalities other than SLE, 8 male and 23 female with an age range of 18 to 63, were compared with SLE group. They included rheumatoid arthritis 7 cases, polyarteritis nodosa 4, chronic thyroiditis 4, myasthenia gravis 3, Sjögren syndrome 3, dermatomyositis 3, juvenile rheumatoid arthritis 1, Behçet's disease 1, ITP 1, ankylosing spondylitis 1 and Weber-Christian's disease 1.

Four antigen solutions for intradermal tests were used 0.1 ml of 5 TU of PPD (Parke, Davis & Co. Detroit, USA), 0.1 ml of 5 u of SK-SD (Lederle, Pearl River, NY, USA), 0.05 ml of 10,000 times dilutes Candida.
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albicans (Hollister-Stier Lab, Dallas, Texas, USA) and 0.1 ml of 1,000 µg/dl solution in phosphate buffered saline of calf thymus DNA (Worthington Biochemical Corp, Freehold, NJ, USA) were intradermally injected on the innerportion of the forearm. The results of the tests were classified as positive or negative according to the transverse diameter of induration at 48 hours. The PPD, SK-SD and DNA intradermal tests were considered positive when there were 10 mm, or more of induration and the Candida tests were positive when the induration was at least 5 mm. 0.1 ml of PBS solution was simultaneously injected as control for excluding the possibility of abnormal reactions by injection procedure.

Peripheral lumphocyte count was obtained from total white cell counts and percentage of lymphocytes at the time of the intradermal tests. Leukocyte migration inhibition test (LMIT), one of the in vitro test for cellular immunity, was done by direct method on agarose plate. Peripheral leukocyte suspensions were devided into 2 groups, consisting of the one incubated with antigen of calf thymus DNA and the other with PBS as control for 30 minutes at 37°C, and then applied to the wells of plate. A migration index was calculated by a ratio of mean migratory areas of leukocytes incubated with antigen to those without antigen as reported previously.

RESULT

Intradermal tests in healthy controls revealed that PPD was negative in one out of 16 persons (6%), SK-SD was negative in 4 of 10 (40%), Candida was negative in none of 16 (0%) and DNA was negative in all of 10 examined (100%). Thus in healthy subjects PPD and Candida were almost positive in most cases, while DNA was negative in all cases.

Fig. 1 showed a correlation between intradermal tests and peripheral lymphocyte counts in 16 cases of SLE, which consisted of active 10 cases and inactive 6 cases. PPD was negative in 14 of 16 (88%), SK-SD was negative in 12 of 14 (86%), Candida was negative in 5 of 14 (36%) and DNA was negative in 9 out of 15 cases (60%). It appeared that cases who were positive for DNA intradermal test predominantly had lymphocytopenia and were clinical active.

![Fig. 1. Intradermal tests in relation to the peripheral lymphocyte counts and disease activity in SLE patients.](image)

Fig. 2 showed a correlation between intradermal tests and LMIT (normal range of 80 to 120%) in 13 cases with SLE consisting of active 8 cases and inactive 5 cases. Clinically active case was found in 4 out of 5 cases (80%) with an abnormal LMIT.
level lower than 80%. Active case was found in 4 out of 8 cases (50%) within normal LMIT range.

Fig. 3 showed a correlation between intradermal tests and peripheral leukocyte counts in 16 SLE cases, dividing into two groups with treatment and without treatment. However, there appeared no correlation among them. Fig. 4 shows the same analysis in 31 cases with other connective tissue diseases, where PPD was negative in 16 of 31 cases (52%), SK-SD was negative in 13 of 31 (42%), Candida was negative in 5 of 31 (16%) and DNA was negative in 14 of 16 (87%). Two cases with positive DNA intradermal test were diagnosed as dermatomyositis and angitis, respectively.

It was statistically significant that negative intradermal tests with PPD, SK-SD and Candida were more frequently seen in SLE cases than in other disorders. It was also statistically significant that positive DNA intradermal test was more frequent in SLE than in others. Leukocytopenia lower than 1,500/mm³ was more frequently seen in SLE (88%) than in others (42%). Leukocytopenia was found randomly among cases irrespective of treatment or nontreatment.

Fig. 5 showed intradermal tests in connection with age. There was no preponderance of negative result of intradermal tests in the aged.

Fig. 3 and Fig. 4. Intradermal tests in relation to the peripheral lymphocyte counts and treatment in patients with SLE and with other connective tissue diseases.

DISCUSSION

SLE is a disease characterized by various immunologic abnormalities. Some studies recently established that both the percentage and absolute number of T-cells of lymphocytes were decreased in SLE. However, reports of intradermal tests on SLE patients appeared in a small numbers of the literature. Abe and Homma performed intradermal tests with PPD in 20 patients with SLE. Four of 20 patients showed
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positive response to PPD, while three quarters of the matched controls had a positive test. This difference was similarly seen in the present study in Figs. 1 and 2. They also applied skin test to DNCB with a result of 35% positive in SLE and 80% positive in controls.

There have been a limited numbers of reports of intradermal test with Candida and SK-SD, among which Horwitz\(^5\) reported that 12 of 14 SLE patients did not develop positive skin reactions, in contrast to most patients with tuberculosis or normal control group. He also suggested that other factors besides reduced numbers of lymphocytes contributed to impaired cutaneous reactivity in SLE in relation of reactivity of intradermal tests to lymphopenic state. Our study favors these results in reference to lymphocyte counts as shown in Figs. 1, 3 and 4.

Another interesting paper was reported by Foad et al\(^6\) cell-mediated immunity was tested in young SLE patients (age range from 20 to 42 yrs), in elderly SLE (age range from 63 to 84 yrs) and in young and elderly control subjects. Evaluation by skin test response to PPD and mumps antigens was not significantly different in these four groups. The area of induration to Candida antigen was significantly less in both groups of elderly patients, suggesting a diminution of cellular immunity with advancing age. Our data showed that negative response to PPD was 6% in normal controls in contrast with 88% in SLE patients, while negative response to Candida was 0% in normal controls in contrast with 36% in SLE patients. One of the reasons for a low incidence of negative PPD in normal of our study may be related to predominance of tuberculosis in the past in oriental countries including Japan. Otherwise the present result appeared to be similar to that by Foad\(^6\).

DNCB skin test is an excellent method for evaluation of cellular immunity. However, patient is to be sensitized with DNCB a few weeks prior to rechallenging, possibly associated with side effects such as cutaneous irritation or erosions. In contrast, skin test to DNA antigen is a simple method without any side effect. DNA tests were evaluated as positive in all 19 patients with SLE and as negative in all 23 control subjects by Ores and Lange\(^7\). Azoury et al\(^8\) carried out intradermal test using calf-thymus DNA with a positive result in 12 of 25 patient with SLE. The 12 patients with a positive reaction had active SLE. Thirty five control subjects were negative to DNA. Fardal et al\(^9\) found the positive DNA intradermal test in 9 of 23-SLE patients and in 31 of 92 dermatological disorders. Our result showed that positive response to DNA was 0% in normal control and 13% in patients with other connective tissue diseases in contrast with 40% in SLE patients. If the concentration of DNA antigen would raise, the result might be more appropriate to evaluate impaired cellular immunity.

Regarding the therapy, corticosteroid greater than 120 mg hydrocortisone-equivalent appeared to have some inhibitory effect on intradermal tests\(^9\). We did not find any correlation between intradermal test and therapy in Figs. 3 and 4.

REFERENCES