mitted illumination with an excitation filter that in addition to blue light also passes red light.

2) The red "autofluorescence" of negative bacteria appears to be the reflection of "unwanted" red excitation light.

3) Fluorescence microscopy with incident illumination cannot be used for the reading of the FLA-ABS test in red and green.

4) Obviously, reading of only FITC fluorescence is quite possible using incident illumination. Phase contrast microscopy or a counterstain for all bacteria might then be used to visualize the negative bacteria as well.

5) With this basic understanding of the differences between transmitted light and incident light in reading the FLA-ABS test, results in terms of specificity and sensitivity should be comparable in the two systems.

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Serological Abnormalities in Japanese Patients with Borderline Leprosy

To the Editor:

It is well known that circulating immune complexes (CIC) and some autoantibodies may be present in sera of patients with lepromatous leprosy ([8, 9, 10]). We have already reported that CIC and antinuclear antibodies (ANA) were present in sera of patients with tuberculoid leprosy as well as lepromatous leprosy ([1, 2]). In spite of the accumulating evidence concerning the appearance of CIC and ANA in lepromatous and tuberculoid leprosy, there are few reports with special reference to CIC and ANA in borderline leprosy.

We collected samples from 25 patients with borderline leprosy (BT, BB, BL) ([11]) in Nagashima Aiseien, Okayama, Japan. The patient ages ranged from 30–72 years, and the clinical stage was regressive ([6]). They were under treatment with antileprosy agents. Patients with other infectious diseases, cancer, and liver diseases were excluded from this study. The Bacteriologic Index (BI) was checked when the blood samples were collected. Twenty-five sera were obtained as controls from healthy individuals whose ages ranged from 25–79 years.

CIC was determined by C1q solid phase assay (SPA) ([4]); the detailed procedures were

THE TABLE. *Serological abnormalities in borderline leprosy.*

	No. examined	C1qSPA[a]	ANA[a]		C3[b]		C4[b]	
			ss	ds	High	Low	High	Low
Borderline leprosy								
Total	25	11 (44)[c]	6 (24)	0	6 (24)	2 (8)	2 (8)	4 (16)
BI positive[d]	15	7 (47)	4 (27)	0	3 (20)	1 (7)	2 (13)	2 (13)
BI negative	10	4 (40)	2 (20)	0	3 (30)	1 (10)	0	2 (20)
Controls	25	2 (8)	4 (16)	0	1 (4)	0	2 (8)	0

[a] Values more than two standard deviations from the mean values of controls were regarded as positive. Cut-off points of C1qSPA, ssDNA antibodies, and dsDNA antibodies were 11 μg/ml, A (405 nm) 0.83 and A (405 nm) 0.97, respectively.

[b] Normal ranges of C3 and C4 were 55–121 mg/ml and 10–55 mg/ml, respectively.

[c] Number in parentheses is percent positive.

[d] BI = Bacteriologic Index.

described previously ([1]). C3 and C4 in sera were measured in single radial immunodiffusion plates (Berringwerke, West Germany). Enzyme-linked immunosorbent assays for antibodies to DNA were carried out according to the slightly modified methods of Kavai, *et al.* ([7]). Briefly, the wells (Dynatech Microelisa plates, West Germany) were coated with 200 μl of methylated bovine serum albumin (mBSA) solution at a concentration of 1 mg/ml in distilled water overnight at 4°C. After washing with phosphate buffered saline (PBS), 100 μl of double-stranded (ds) DNA (50 μg/ml) and single-stranded (ss) DNA (50 μg/ml), for which isolation procedures and specificity were described previously ([2]), were added to wells. For the control wells, 100 μl of PBS–1 mM EDTA·2Na was added. After incubation for 3 hr at room temperature (RT) and washing, heat-inactivated serum samples were diluted with PBS–1 mM EDTA·2Na–0.05% Tween 20, 1:10, and 100 μl of each was measured into the wells. The plates were then incubated for 1 hr at RT, and washed with PBS. For measurement of IgG-anti-DNA, protein A conjugated with alkaline phosphatase (Sigma Chemical Co., St. Louis, Missouri, U.S.A.) prepared in our laboratory was used in a dilution of 1:400. The wells were incubated with 100 μl of the conjugate for 1 hr at RT. After washing, 200 μl of p-nitrophenylphosphate (0.4 mg/ml in diethanolamine buffer, pH 9.8) was added to the wells. The color was measured in a Titertek Multiskan (Flow Laboratories, Inc., Rockville, Maryland, U.S.A.) at 405 nm. Anti-DNA levels were expressed as follows: (Anti-DNA level) = (absorbance of DNA-coated wells) − (absorbance of wells not coated with DNA). Anti-DNA levels more than the mean plus two standard deviations (S.D.) of the normal controls were considered as positive.

The results of the serological tests are summarized in The Table. Compared with controls, the CIC levels were significantly high but the other serological tests were within normal limits. CIC were demonstrated in 11 patients (44%) with borderline leprosy, which was similar to our previous report ([1]). However, qualitative and quantitative differences were not present between the BI-positive group and the BI-negative group. A few investigators have reported the presence of CIC in borderline leprosy using C1q precipitating tests ([3]) and platelet aggregation tests ([13]). These reports showed that 15%–25% of the patients had CIC in their sera. The increased incidence in the present study might be due to the increased sensitivity of the C1qSPA. ANA in borderline leprosy have been considered to be absent by some workers ([14]). In preliminary work, we determined qualitative ANA in sera of patients by using an indirect immunofluorescent technique in which the serum dilution of 1:10 and cultured fibrocytes (Meloy Laboratories, Inc., Springfield, Virginia, U.S.A.) were used as substrates. Five patients (20%) showed positive but weak reactions against nuclear antigens with a homogenous speckled pattern. Furthermore, as shown in The Table, ANA seemed to react against single-stranded DNA. Anti-ds DNA antibodies were absent in the sera of both the patients and the controls. C3 and C4 in the sera of patients showed a

normal distribution, although high or low levels of complements were observed in some patients. These results were compatible with recent investigations by Iliadi-Alexandrou, *et al.* ([5]).

In general, abnormalities of humoral immunity have been more frequently observed in lepromatous leprosy than in other forms ([1,2]). Most recently we studied CIC and ANA in lepromatous and tuberculoid leprosy. CIC were demonstrated in about 50% of the patients in both of the polar forms, and anti-ss DNA antibodies were found in over 20% of the patients with lepromatous leprosy and 9% of the patients with tuberculoid leprosy. Furthermore, statistical analysis revealed no significant differences between lepromatous leprosy and tuberculoid leprosy ([2]). Based on our previous results, the positivity rates of CIC and ANA in borderline leprosy were similar to those in lepromatous leprosy and tuberculoid leprosy. This accumulating evidence suggests that the presence of CIC and ANA in sera are common findings in all forms of leprosy and, therefore, it seems to be impossible to discriminate among the forms of leprosy by these humoral abnormalities.

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