Immunochemical and Serologic Patterns in Leprosy

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An infectious disease of chronic character should be expected to give rise to antibody formation. Cellular antibodies in tuberculoid leprosy can be demonstrated by means of the lepromin test. Circulating antibodies, however, have not been demonstrated with specific antigens, because of the fact that up until now it has not been possible to grow the mycobacteria of leprosy in a sufficient amount to produce an antigen from the bacteria.

Extracts from other mycobacteria have been used as antigens, and these extracts have to a certain extent been usable for the demonstration of circulating antibodies in leprosy. From human tubercle bacilli and from certain BCG strains, Takahashi has isolated a phospholipid which has proved to be usable for detecting circulating antibodies in tuberculosis when used in a kaolin-precipitation test. The antigen developed by Takahashi has also been found to be reactive in leprosy. In our laboratory we have found Takahashi's antigen highly sensitive when used in a complement-fixation test for leprosy after addition of cholesterol to the original antigen.

For a long time it has been known that sera from leprosy patients in some cases have shown reactivity with syphilis antigens, even if the patients had never suffered from syphilis. For many years such reactivity was named "biologically false positive," even if there was nothing "false" in the reactivity. During the last 20 years the TPI test has been in use as a verification-test for positive sero-reactions found with lipid antigens; "nontreponemal," therefore, is a better designation for the reactivity occurring in non-syphilitics. Sera from leprosy patients, especially from patients with lepromatous leprosy, have been found reactive to a high extent, not only with respect to syphilis antigens, but also in many other serologic procedures, e.g., ANF, thyroglobulin antibody and rheumatoid arthritis factor, among others Matthews & Trautman ([4]).

The crude extracts from oxhearts, previously used in the serologic diagnosis of syphilis, were, in the late 1940's, replaced by cardiolipin antigen. The cardiolipin antigen turned out to be more specific than the crude antigen, even if nontreponemal reactions also occurred with cardiolipin antigen.

The following is a report of my examination of leprosy sera with cardiolipin antigen in various combinations. A total of 500 sera from leprosy patients in leprosaria in Egypt, India and Malaysia form the basis for the present communication ([8, 9]). The sera originated mainly from patients suffering from the polar forms of leprosy; only a very few sera were from cases of intermediate leprosy.

Due clinical information was obtained from doctors in the leprosaria. In a small group of patients lepromin-testing was done at the time of blood-testing. The clot was removed before shipment, and pure serum was then mailed to my laboratory in nonheated state without addition of any preservatives. All samples were air-mailed and afterward heat-inactivated at 56° Celsius for half an hour. A total of 10 sera have been examined immunochemically for their content of IgG, IgA and IgM. The immunochemical examinations were made by Dr. Jørgen Clausen, Neurokemisk Institut, Copenhagen.

ANTIGENS

Sera were submitted for examination in the flocculation reactions VDRL, KR and MR, and in two complement-fixation tests. In one of the complement-fixation tests, CWRM, ordinary cardiolipin antigen in a mutual combination of cardiolipin, lecithin and cholesterol in the ratio 1:5:17, as recommended by Maltaner and Maltaner ([3]), was used. In another complement-fixation test an antigen composed of cardiolipin and cholesterol in the ratio of 1:17 without any

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lecithin was used ([6, 7]). From previous experiments, e.g., Ogata ([5]), Kent, Otero and Harrigan ([2]), Kent, Burke, Carroll and Simonson (1958) ([1]), it was known that the concentration of lecithin influences the sensivity as well as the specificity of cardiolipin antigen. Increase of lecithin-concentration to a certain extent will give an antigen which is more specific, i.e., gives a smaller number of nontreponemal reactions.

As mentioned, all sera were examined simultaneously with these two antigens in complement-fixation tests. Most of the sera were examined in TPI, too, in order to evaluate if any of the lipoid-positive sero-reactions might be treponemal. From Table 1, which covers the whole material of sera from patients suffering from lepromatous leprosy, it will be seen that the reactivity with lecithin-free cardiolipin antigen, Cardchol, amounts to approximately 60 per cent, and to a reactivity of approximately 20 per cent with cardiolipin antigen of "normal" composition. It is furthermore shown that the combination CWRM-reactive–Cardchol-nonreactive is nonexistent, whereas 78 sera were reactive with Cardchol and nonreactive in CWRM. Finally, 36 sera, i.e., about one-fifth, were reactive with both antigens from complement-fixation reactions. In this table no regard is taken as to duration of disease or treatment, but the diagnosis was, according to doctors' information, lepromatous leprosy in all cases.

TABLE 1. *Reactivity of CWRM and cardchol respectively for 193 sera from patients suffering from lepromatous leprosy.*

CWRM	Cardchol	Number
−	−	79
+	−	0
−	+	78
+	+	36

Cardchol reactive: 59%
CWRM reactive: 18%

Table 2 shows the reactivity in the complement-fixation tests, put into relation with the reactivity of the flocculation reactions VDRL, KR and TPI. From the table it is seen that there is some reactivity in the flocculation reactions. Furthermore, this reactivity was increasing simultaneously with increasing reactivity in the complement-fixation reactions, but not to the same extent as that of the complement-fixation tests. Only five of the examined 161 sera turned out to be reactive in TPI, and thus regarded as originating from syphilitics.

In Table 3 the material is subdivided according to the duration of disease, and it is seen that the reactivity of sera seems to decrease with duration of the disease. This may be due to the effect of treatment, and may be visualized as parallel to the conditions in syphilis, viz., a decrease of antibody content with time, and then a new increase of titer by renewed activity. As the material examined was relatively small,

TABLE 2. *Number of sera in each of four possible combinations of CWRM and Cardchol and reactivity of sera in each group in the examinations with KR, VDRL and TPI tests.*

Complement-fixation tests		Number of sera also positive in other tests		
Results	Number of sera	KR+	VDRL+	TPI+
CWRM −/Cardchol −	82	1	5	2
CWRM +/Cardchol −	0	—	—	—
CWRM −/Cardcdol +	62	9	8	1
CWRM +/Cardchol +	17	8	5	2
Total	161	18	18	5

TABLE 3. *Reactivity of CWRM and Cardchol according to duration and type of leprosy.*

Duration of leprosy	Reactivity			
	CWRM −/ Cardchol −	CWRM −/ Cardchol +	CWRM +/ Cardchol +	Total
	Tuberculoid leprosy			
< 20 years	29 (71%)	10	2	41
> 20 years	16 (94%)	1	0	17
Total	45	11	2	58
	Lepromatous leprosy, negative smears			
< 20 years	17 (49%)	12	6	35
> 20 years	3 (60%)	1	1	5
Total	20	13	7	40
	Lepromatous leprosy, positive smears			
< 20 years	14 (26%)	33		54
> 20 years	2 (33%)	3	1	6
Total	16	36	8	60
Total patients				158

division in two groups, below and over 20 year's duration of disease, was the only procedure possible.

As mentioned before, the lepromin test was performed on a smaller number of patients at the time of blood testing. Table 4 shows the serologic results and the results of the lepromin test, respectively. Thirty patients were lepromin-negative, and 11 lepromin-positive. Out of the 30 lepromin-negative patients 18 showed serologic reactivity, which equals 60 per cent of the examined patients, whereas only two out of 11 lepromin-positive patients showed reactivity in the complement-fixation tests. Thus there seems to exist a negative correlation between cellular and circulating antibodies. The difference between 18 out of 30 and two out of 11 is statistically significant at a 0.2 per cent level.

In previous communications many authors have postulated that the incidence of syphilis in leprosy patients was so high that the serologic reactivity in leprosy might be due to syphilis. On performing TPI on all sera we found a reactive TPI in 19 per cent of the sera from India, and a reactive TPI in 3 per cent of the sera from Egypt. These figures exclude the possibility that the positive sero-reactions with the cardiolipin-antigens in leprosy could be caused by syphilis, and at least the percentage of syphilis for the Egyptian material is in accordance with the percentage of syphilis in the population as a whole.

Immunochemical examinations of 10 sera from patients suffering from lepromatous leprosy in Malaysia have not revealed changes of a constant pattern. On immuno-

TABLE 4. *Relationship between reactivity in lepromin test and reactivity in CWRM and Cardchol tests.*

CWRM/ Cardchol	Result of lepromin test		Total
	−	+	
− −	12	9	21
+ −	0	0	0
− +	11	1	12
+ +	7	1	8
Total	30	11	41

chemical examination IgG was found increased in two, decreased in four, and six, but increased in four, and finally, IgM was increased in five, reduced in one, and normal in four patients. These results do not warrant any final conclusion as to the immunochemical pattern, and the tests ought to be done on a bigger material of sera.

SUMMARY

The serologic examinations, as noted here, do not justify a claim that cardiolipin antigen, even not in the lecithin-free form, detects antibodies specific for leprosy. The results must be regarded in the light of the ability of leprosy sera to react in various antigen-antibody procedures. It is lepromatous leprosy especially which gives rise to a stimulation of the antibody-formation, and in this capacity lepromatous leprosy seems very much like collagen diseases. The reactivity of cardiolipin antigen in leprosy, demonstrating the presence of antilipoid antibodies; should be regarded by analogy with syphilis and collagen diseases, where the serologic reactivity has been interpreted as a result of tissue destruction.

The decreasing reactivity observed when leprosy has lasted for a longer period might express a minor activity of the infection. On the assumption that treatment influences the activity, serologic examinations might be helpful in judging the prognosis and response of various treatments. Finally it should be noted that the reactivity observed in our laboratory presumably is a minimum, because shipment, including storage for a longer period of time, of sera with nontreponemal activity has been shown to reduce the titer ([10, 11]).

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