Antigenic Properties of Some Mycobacterial Strains Isolated and Cultivated From Leprosy Tissue

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As we have described in previous papers ([1, 2, 3, 4]) mycobacteria produced growth in only 16 percent of the cases when isolated from lepromatous tissue, and very rarely from tuberculoid or dimorphous tissue. Strains growing on Loewenstein-Jensen medium at about 32°C. could be divided into the three groups I, II or III. Nonphotochromogenic mycobacteria (Runyon III) demonstrated pathogenicity in white rats. After intratesticular injections we could observe remarkable alterations of the testis. In specimens stained by the Faraco-Fite method numerous mycobacteria, sometimes in globus-like aggregations, were to be seen. Without great difficulty these germs were isolated and cultivated from the rats' testes. In rats infected intradermally only small skin infiltrations were registered. In hamsters some of the tested strains, when administered intracutaneously, caused nodules at the site of the injection. These alterations did not lead to further generalization.

In this paper we shall report our findings with respect to the agglutination properties of some strains in blood sera from leprosy and nonleprosy patients.

MATERIALS AND METHODS

Patients. We investigated the agglutination properties of blood sera of 21 leprosy patients (LL, 14 cases; LD 4; LT 1, and LI 2), turning to the use of different antigens. In addition 21 blood sera of nonleprosy patients (10 cases of open pulmonary tuberculosis and 11 patients with skin diseases of different etiology) have been tested in the same manner.

Antigens. We employed the following antigens, which we had prepared in our laboratory:

1. "A-M.A.-I": A mycobacterial strain isolated from leprosy tissue in Caracas.
2. "A-Zulia"-II": Strain isolated from leprosy tissue in Maracaibo.
3. "A-P-III": Strain isolated from dimorphous tissue in Caracas.
4. "a-My-I": *M. avium*, type collection strain.
5. "b-My-II": Scotochromogenic strain (Runyon II) isolated from the sputum of a tuberculous patient in Maracaibo.
6. "c-My-III": A nonphotochromogenic strain (Runyon III), isolated from the sputum of a tuberculous patient in Maracaibo.

We prepared the antigens as follows: To a well homogenized bacterial infusion in 0.85 per cent salt solution (5-8 mgm. of moist weight, centrifuged and washed, to 1 ml. liquid) we added 0.4 per cent Formol (35% formaldehyde by volume). This suspension was kept in an incubator for two days at 30°C. Thereafter we added 4–6 drops of a 0.6 per cent brilliant-green solution to 10 ml. of antigen. The whole solution was shaken and filtered through a 7-fold sterile gauze.

Reaction Methods. We employed the rapid agglutination method and observed reactions on the plate of a Huddleson's box. The serum in quantities of 0.04, 0.02, 0.01 and 0.005 ml. was added by pipet to the plate and mixed with a drop (0.03 ml.) of the homologous antigen. The results were read within five minutes. We took into consideration only complete agglutinations, when a peripheral agglutination ring could be observed at the point of the drop mixture. The data are summarized in Tables 1 and 2.

RESULTS

From Table 1 we can gather that the serum of leprosy patients in 17 of the 21 tested cases could agglutinate, nearly always up to smallest serum quantities, the

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TABLE 1. *Rapid agglutination tests in vitro with 6 different antigens and blood sera of leprosy-patients. Volumes of sera: 0.04, 0.02, 0.01 and 0.005 ml.*

No.	Patient	Leprosy type[a]	Antigen					
			I	II	III	a	b	c
1	F.T.	LD	—	—	XXXX	—	—	—
2	M.G.B.	LD	—	—	XXXX	—	—	—
3	V.P.	LL	XXX	XXXX	—	—	—	—
4	M.C.	LL	—	—	XXXX	—	X	—
5	P.C.	LL	XXXX	—	XX	—	—	—
6	B.A.	LL	XXXX	—	XXXX	—	—	—
7	J.A.L.	LI	—	—	—	—	—	—
8	A.J.O.	LI	XXXX	—	—	—	—	—
9	P.M.	LT	XXX	—	—	—	—	—
10	A.A.A.	LL	—	—	XXXX	—	—	—
11	J.D.	LL	—	—	—	—	—	—
12	M.C.V.	LL	XXX	—	XXXX	—	—	—
13	P.R.	LD	—	—	XX	—	—	X
14	P.C.	LL	XXXX	—	X	—	—	—
15	C.A.	LD	—	XX	XXXX	—	—	—
16	A.V.	LL	XXX	XXXX	—	—	—	—
17	J.M.	LL	XXX	—	XXX	—	—	—
18	B.O.	LL	XXX	—	—	—	—	—
19	R.Ch.	LL	XXXX	XXXX	XXXX	—	—	—
20	J.V.M.	LL	—	—	—	—	—	—
21	J.D.	LL	—	—	—	—	—	—
Rat sera			—	—	—	—	—	—

[a] LL, lepromatous; LD, dimorphous; LT, tuberculoid; LI, indeterminate.

mycobacteria isolated from leprosy tissue. It is worth mentioning that in one case only (No. 19) all three antigens (I, II and III) had agglutinated. In 8 cases (Nos. 3, 5, 6, 12, 14, 15, 16 and 17) only two showed reactions with antigens I and II (case Nos. 3 and 16). In case Nos. 5, 6, 12, 14 and 17, with antigens II and III, and in case No. 15 with antigens II and III, and in an additional 8 cases, only one antigen showed an effect (case Nos. 8, 9 and 14) with antigen I, and case Nos. 1, 2, 4, 10 and 13 with antigen III. The remaining three antigens: a (*M. avium*), b (Runyon II) and c (Runyon III) proved to be completely inactive, with the exception of discrete agglutinations (cases Nos. 4 and 13).

Table 2 shows agglutination tests with sera of nonleprosy patients. From this table we can see that sera from nonleprosy patients could not agglutinate antigens I and II from leprosy tissue in any of the 21 cases tested. With antigen III from the dimorphous tissue positive reactions could be demonstrated in three cases, viz., Nos. 1, 17 and 20, at serum volumes of 0.04 ml., and in one case (No. 7) at a volume of 0.01 ml. The remaining antigens (a, b and c), with the exception of c (Runyon III), which showed reactions in case Nos. 1, 7, 8 and 12, proved to be completely inactive.

CONCLUSION

The agglutination tests cited above demonstrate that mycobacteria isolated from leprosy tissue react significantly with sera of leprosy patients in 80.9 percent of

TABLE 2. *Rapid agglutination test* in vitro *with 6 different antigens and blood sera of non-leprosy patients. Volumes of sera: 0.04 and 0.01 ml.*

No.	Patient	Diagnosis	Antigen					
			I	II	III	a	b	c
1	I.F.	Pulmonary tuberculosis	—	—	x	—	—	x
2	R.G.	Pulmonary tuberculosis	—	—	—	—	—	—
3	M.B.	Pulmonary tuberculosis	—	—	—	—	—	—
4	E.M.	Pulmonary tuberculosis	—	—	—	—	—	—
5	L.N.	Pulmonary tuberculosis	—	—	—	—	—	—
6	M.V.	Pulmonary tuberculosis	—	—	—	—	—	—
7	M.P.	Pulmonary tuberculosis	—	—	xxx	—	—	xxx
8	H.J.G.	Pulmonary tuberculosis	—	—	—	—	—	x
9	A.G.	Pulmonary tuberculosis	—	—	—	—	—	—
10	J.G.	Pulmonary tuberculosis	—	—	—	—	—	—
11	D.J.U.	Skin tuberculosis	—	—	—	—	—	—
12	J.J.O.	Syphilis	—	—	—	—	—	x
13	M.V.	Erysipelas	—	—	—	—	—	—
14	J.A.S.	Favus	—	—	—	—	—	—
15	M.de V.	Urticaria	—	—	—	—	—	—
16	A.E.M.	Vitiligo	—	—	—	—	—	—
17	C.A.	Dermatitis	—	—	x	—	—	—
18	A.V.	Psoriasis	—	—	—	—	—	—
19	R.V.	Dermatitis	—	—	—	—	—	—
20	D.M.	Pitiriasis alba	—	—	x	—	—	—
21	M.J.C.	Dermatitis	—	—	—			

Explanation of symbols to Tables 1 and 2:

I Strain isolated from leprosy tissue (M.A.-Caracas).
II Strain isolated from leprosy tissue (Zulia-1).
III Strain isolated from dimorphous-leprosy tissue (Pa-Caracas).
a *M. avium.*
b Runyon II, strain isolated from sputum of a pulmonary tuberculous patient.
c Runyon III, strain isolated from sputum of a pulmonary tuberculous patient.
— No agglutination.
x Positive agglutination-reaction at a serum volume of 0.04 ml.
xx Positive agglutination reaction at a serum volume of 0.04 and 0.02 ml.
xxx Positive agglutination reaction at a serum volume of 0.04, 0.02 and 0.01 ml.
xxxx Positive agglutination reaction at a serum volume of 0.04, 0.02, 0.01 and 0.005 ml.

all cases. In the same tests we discovered that the sera of nonleprosy patients including pulmonary tuberculosis and skin diseases will show equal reactions in only 14 per cent of the cases tested. Contrary to nearly all other pathogenic mycobacteria which *in vitro* form almost no humoral antigens, we observed a phenomenon which offers new possibilities in the field of immunology and especially in leprosy research. It is obvious that the mycobacteria isolated from leprosy tissue demonstrate no serologic similarities with each other, and that their ability to react with the same sera reveals qualitative and quantitative differences. For this reason we can suppose that other similar strains may exist which can react positively as specific agglutinogens with the sera of leprosy patients. Therefore we believe that the strains tested (I, II and III) belong to different serotypes, which nevertheless have the same biologic connection, also in combination, with leprosy patients.

SUMMARY

Sera from a total of 21 leprosy patients were tested for their possible agglutination capacity by the method of rapid-agglutination, with three mycobacterial strains (antigens I, II and III), obtained from three human leprosy lesions cultured in Loewenstein-Jensen media. As controls, sera from 21 nonleprosy patients (10 with pulmonary tuberculosis and 11 with other dermatologic diseases), were tested with the same system. In addition, three other antigens were used (antigens a, b and c): one, *M. avium*, and two chromogens (Runyon II and III), which were isolated in the culture of sputum of tuberculous patients.

Agglutination ests showed that mycobacterial cultures from leprosy tissue led to definite positive reactions in 17 out of the 21 leprous patients tested (in one case all 3 strains, in 8 instances 2 strains and finally in 8 cases only one antigen). The other antigens used as controls (*M. avium*, Runyon II and III) were practically inactive. In the group of 21 nonleprous patients, there were four positive seroagglutination reactions (antigen III).

The agglutination phenomenon here described is in contrast to results with almost all other pathogenic mycobacteria, which have a kind of agglutinogen that forms hardly perceptible agglutinins. This finding opens new possibilities in the field of leprosy research.

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