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Antigens of *Mycobacterium leprae* in urine during treatment of patients with lepromatous leprosy

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Summary Eighteen lepromatous leprosy patients were studied for urine *Mycobacterium leprae* antigen excretion during effective treatment. The amounts of antigen excreted varied during treatment and were in most cases decreasing during effective treatment.

Introduction

In leprosy the clinical changes in skin and nerves are slowly developing, except in reactional situations. During successful treatment the signs and symptoms are also slowly changing and many weeks to months may be required before improvements can be noted. In order to be able to follow the slow changes, drawings and/or photographic methods can be used for recording the skin changes; the bacteriologic index (BJ) and morphological index (MI) in slit-skin smears followed; the degree of nerve enlargement described and the motor, sensory and vegetative nerve functions documented with, for example, drawings, voluntary muscle testing and studies of nerve conductivity.[1]

In a previous paper the presence of *Mycobacterium leprae* antigen in the urine from most patients with lepromatous leprosy was documented.[2] This finding has initiated the formulation of questions like: can the amounts of antigen excreted in the urine be a new way to follow a patient with leprosy?; can antigen detection in urine be an early sign of relapse?; can it be found before clinical signs and symptoms have emerged?; can it be used in epidemiological surveys? etc.

The present study was subsequently conducted in order to see if the amounts

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of *M. leprae* antigens excreted in the urine per 24 hr was changing during the treatment of patients with lepromatous leprosy.

Material and methods

PATIENTS

Twenty patients with lepromatous leprosy (8 with BL and 12 with LL), admitted to the hospital of the All Africa Leprosy and Rehabilitation Training Centre (ALERT) between October 1982 and November 1983, were included, Some background data for these patients are presented in Table 1.

The patients were diagnosed and classified on the basis of clinical, bacteriological and in most cases histopathological examination. The WHO-recommendations for multidrug therapy of lepromatous leprosy[3] were in the process of being started but were not introduced until the study was finished.

Ten patients were newly diagnosed and untreated while two had been treated previously with 100 mg DDS daily for less than 1 year and then stopped taking the drug.

Eight patients had been on 100 mg DDS daily for many years and were hospitalized because of increased signs and/or symptoms of the disease, suggesting the presence of DDS-resistant bacilli and/or irregular DDS intake. Seven of these patients were therefore started on supervised full dosage DDS and closely followed up. Clinical and bacteriological examination confirmed DDS resistance in three of the eight patients (Nos 111, 112 and 113). Their treatment was consequently changed to clofazimine 100 mg with an initial 3 weeks of rifampicin 600 mg daily.

The average duration prior to the start of this study of leprosy in the untreated patients was $2\frac{1}{2}$ years and for the DDS-treated patients 12 years.

URINARY SPECIMENS

The 24-hr production of urine was collected on one or two occasions before the start of treatment for the 10 newly diagnosed patients and the two who had previously been treated by DDS, and while on continued DDS-treatment for the eight suspected DDS-resistant cases. Samples of 24-hr urine were collected on several occasions after initiation or change of treatment. The sampling was scheduled to take place after 3 to 4 days and after 1, 2, 3, 4, 8 and 16 weeks. In some cases additional samples were taken on later occasions.

The 24-hr urine production was collected in clean beakers with 10 ml of NaN_3 (100 mg/ml of double-distilled water) added in advance. The volume of the collected urine was measured. After storage over night at $+4°C$ 300 ml was centrifuged at 17·000 *G* for 20 min and concentrated 10 times in an ultrafiltration

Table 1. Some basic data for the lepromatous leprosy patients studied

Patient no.	Identification	Sex	Age (years)	Classification	Duration of leprosy (years)	Previous treatment	MFP-test†	Treatment after initial urine sampling‡
95	DLO 2714	M	36	LL	2	0	LR or S	DDS + Rif. + Clof. + Eth. (6w) → DDS + Clof.
98	GBO 3297	M	41	LL	8	DDS	—	DDS (4w) → DDS + Rif. (3w) → DDS
100	ATO 426	F	34	LL	2	0	LR	DDS (4w) → DDS + Rif. (3w) → DDS
102	11788	M	46	LL	31	DDS	MR	DDS (4w) → DDS inj (4w) → DDS
103	SGO 7699	M	22	LL	3	0	LR	DDS + Rif. + Clof. + Eth. (8w) → DDS + Clof.
106	STO 7722	F	30	LL	10	DDS	—	DDS
108	SGO 7728	M	15	LL	1	DDS-0*	—	DDS + Rif. (3w) → DDS
109	BGO 1201	F	49	BL	10	DDS	MR	DDS
110	SGO 7752	M	29	LL	8	DDS	LR	DDS
111	STO 1514	M	35	BL	15	DDS	MR or HR	DDS (4w) → Rif. + Clof.(3w) → Clof.
112	STO 7766	M	51	LL	10	DDS	HR	DDS (8w) → Rif. + Clof. (3w) → Clof.
113	SMO 771	M	45	BL	18	DDS	HR	Rif. + Clof.(3w) → Clof.
114	SJO 7774	M	15	BL	2	0	—	DDS + Rif. (3w) → DDS
115	SMO 7780	F	16	BL	1	0	—	DDS + Rif. (3w) → DDS
116	SMO 7792	F	26	LL	2	0	—	DDS + Rif. (3w) → DDS
117	GDO 3330	F	18	LL	1	0	—	DDS + Rif. (1d) → DDS
118	SGO 7817	M	45	LL	8	0	LR	DDS + Rif. (3w) → DDS
119	SJO 7819	M	28	BL	2	0	S	DDS + Rif. (3w) → DDS
120	SJO 7820	M	18	LL	5	DDS-0*	—	DDS + Rif. (3w) → DDS
121	SJO 7821	F	12	LL	3	0	S	DDS + Rif. (3w) → DDS

* Short time DDS, but untreated on admission

† Results of mouse footpad tests. S, sensitive; LR, low resistant; MR, medium resistant; HR, highly resistant; —, not performed.

‡ Rif., rifampicin 600 mg/d; Clof., clofazimine 100 mg/d; Eth, ethionamide 500 mg/d. In brackets the period of treatment in weeks (w), or days (d) are noted.

cell with a Diaflo ultrafilter YM10 (cut-off 10·000 MW) as previously described.[2] The concentrate was lyophilized and the dry powder dissolved in double-distilled water to a volume corresponding to 1 : 100 the original and stored at −20°C until assayed.

RADIOIMMUNOASSAY (RIA) FOR M. LEPRAE ANTIGEN

An inhibilitory RIA-test has earlier been worked out and described.[2] In this test the presence of *M. leprae* antigen in a sample inhibits the binding of radiolabelled *M. leprae* antigen to a given antibody. The antibodies and their antigens are subsequently bound to a solid phase (Cowan I staphylococci = CI) and pelleted before being counted. High gamma-radioactivity in the pellet means that low amounts of *M. leprae* antigen are present in the sample, and low radioactivity that higher quantities of *M. leprae* antigen are present.

The concentrated urine samples were thawed and assayed for *M. leprae* antigen with a small modification compared to the method earlier described.[2] In short, a preparation of sonicated armadillo derived *M. leprae* was labelled with ^{125}I by electrolytic iodination (^{125}I-AM1S). In the RIA the urine samples were run in duplicate in disposable polyethylene tubes (Koch-Light Laboratories Ltd, England). Each tube contained 50 μl of a 10^{-3} dilution of a selected serum from a patient with BT leprosy containing high titres against mycobacterial arabinogalactan (AG) and arabinomannan (serum no 625/78 in ref. 4), but low titres against other mycobacterial antigens present in the ^{125}J-AM1S preparation. Fifty microlitres of concentrated urine was added after absorbing 100 μl of the urine with 20 mg net weight formalinized *Staphylococcus aureus* strain Cowan I (Pharmacia Diagnostics AB, Sweden) for 5 min at room temperature. The absorption was done in order to get rid of anti-*M. leprae* antibodies in the urine which interfered with the RIA. This procedure may also absorb antibodies complexed with the corresponding antigens resulting in antigen loss. CI is also known not to bind IgG 3. If anti-*M. leprae* antibodies of this subgroup stay in the urine they may bind radioactive ^{125}I-AM1S and by this give a lowered final count like the effect of an antigen. Fifty microlitres of 0·025 M EDTA in double-distilled water were added to bind urine Ca-ions which had been observed to precipitate ^{125}I-AM1S preparations. After 30 min incubation with 50 μl of ^{125}I-AM1S at room temperature Cowan I staphylococci (1 ml of a 1% suspension in PBS with 1% Tween 20) was added. By this procedure the antibodies in serum 625/78 with antigen from either the urine sample or ^{125}J-AM1S were bound to the staphylococci. The staphylococci were pelleted, the supernatant aspirated and the pellet counted in a LKB-Wallac 1270 Rackgamma II gamma counter. Dilutions of a crude *M. tuberculosis* derived AG preparation were used as a reference antigen for the quantification of urine antigen. Samples from two of the 10 untreated patients (Nos 95 and 114) had very high anti-*M. leprae* antibody-like activity which was not fully inactivated by absorption with C1 and heat

treatment, and could therefore not be analysed for *M. leprae* antigen with the presently used RIA.

MOUSE FOOTPAD (MFP) TEST FOR SENSITIVITY TESTING OF M. LEPRAE TO DDS.

The MFP test was performed with *M. leprae* from 12 patients as previously described[5,6] using 0·0001%, 0·001% and 0·01% of DDS in the food of locally bred Swiss albino mice, six in each group. A group of six mice without DDS in the food served as control. The sensitivity to DDS was reported as sensitive, low resistant, medium resistant or highly resistant (resistant to 0·0001, 0·001 and 0·01%, DDS respectively).

Results

In order to get data on the variations of the amounts of *M. leprae* antigen excreted in urine, sampling was performed for two 24-hr periods on five patients before the start of DDS-treatment and on eight patients who continued their daily DDS-treatment. The results are summarized in Table 2. In all the patients the difference

Table 2. The amounts of *M. leprae* antigen excreted in the urine during two 24-hr periods before the start of treatment or while still continuing DDS 100 mg daily over a period of many years

Patient No.	Previous treatment	Amount of *M. leprae* antigen (μg) in 24 hr urine	
		(period 1)	(period 2)
98	DDS	670	270
100	0	< 20	< 20
102	DDS	< 20	110
103	0	2300	500
106	DDS	< 20	36
108	DDS-0	370	560
109	DDS	22	47
110	DDS	20	49
111	DDS	50	38
112	DDS	430	910
113	DDS	38	86
118	0	< 20	150
120	DDS-0	5500	7000

between the samples was less than tenfold. The ambition to follow untreated lepromatous leprosy patients for longer times before start of treatment was considered to be unethical.

In Table 3 the amounts of *M. leprae* antigen in 24 hr urine at different times after start of treatment of 10 patients who were not on treatment on admission are presented. The two patients (Nos 100 and 116) without detectable *M. leprae* antigen in urine (the detection level was 20 μg of antigen in a 24-hr urine sample) on admission both got detectable antigen in the urine after start of treatment. The amounts of antigen excreted by these two patients were comparatively low with a maximum of 220 μg/24 hr for No. 116. In patient No. 103, who was treated with four drugs, the antigen excretion steadily decreased during the treatment and became negative after 8 weeks. Seven patients had the highest measured amount of antigen excreted after 1 week and/or 4 weeks of treatment. Two of these patients had however not been able to follow more than 4 weeks and any maximum of excretion at this time can thus not be suggested. The treatment was effective clinically and accompanied by falling MI in all except one patient (No. 100) the treatment of whom was changed after 4 weeks.

Seven patients, previously treated with DDS 100 mg daily and suspected to harbour DDS-resistant bacilli, were admitted for supervised DDS treatment (100 mg daily). The results of tests for *M. leprae* antigen excretion in urine during this treatment is documented in Table 4. Two patients (Nos 111 and 112) did not respond clinically and the MI did not fall. Results of the MFP studies showed their *M. leprae* to be medium and highly resistant to DDS respectively. The antigen excretion stayed within the same magnitude for these two patients during the supervised therapy. Patient No. 98 was treated by supervised DDS 100 mg daily for 4 weeks. The amounts of *M. leprae* antigen in urine were on the same level during this treatment as before supervised treatment. The patient improved clinically and MI fell from 3·2 to 0·5% during this period. Rifampicin 600 mg daily was then added for 21 days (Table 5). A peak of antigen excretion was then seen on the last day of rifampicin and the urine was negative 45 weeks later. Four patients (Nos 102, 106, 109 and 110) improved clinically on supervised DDS treatment and were continued on this drug. Three of them turned negative for urinary *M. leprae* antigen, the fourth was not followed for more than 8 weeks when he was still positive.

The amounts of urine *M. leprae* antigen from patients who previously were treated by DDS and got additional rifampicin 600 mg daily for 21 days or who were treated by clofazimine 100 mg daily plus rifampicin are presented in Table 5. During these new drug-regimens all the patients improved clinically and had falling MI values. The addition of rifampicin to DDS in patient No. 100 resulted in detectable levels of antigen excretion after 3 and 4 weeks like it did 1, 2 and 3 weeks after starting on DDS monotherapy (Table 3).

Three patients with proven DDS resistance were treated by clofazimine and rifampicin (Nos 111, 112 and 113). In these patients the antigen excretion also

Table 3. The amounts of *M. leprae* antigen excreted in the urine before and after initiation of drug treatment in previously untreated patients

Patient No.	Initial treatment of patients without drugs on admission	Amounts of *M. leprae* antigen before *and* after initiation of drug treatment								
		before*	3–4d†	1w†	2w	3w	4w	8w	16w	32w
100	DDS	< 20	< 20	220	82	26	< 20			
103	DDS + Rif. + Clof. + Eth.	1400	1300		1200	700		< 20	< 20	
108	DDS + Rif.	465	810	570	120	51	670			
115	DDS + Rif.	30		< 20	< 20	23		55	180	
116	DDS + Rif.	< 20	72	47	90	45	210	150	120	20
117	DDS + Rif.	860	380	≥ 7000	1200	5200	≥ 7000	100	310	
118	DDS + Rif.	75	< 20	300	62	21	280			
119	DDS + Rif.		420	7000		1000	63			
120	DDS + Rif.	6250	400	670	1500	≥ 7000		860	710	
121	DDS + Rif.	2700	≥ 7000	1400	≥ 7000	≥ 7000	2500	≥ 7000		

* μg of *M. leprae* antigen in 24-hr urine. Results of single sample or average of repeated samples.
† d = days, w = week(s) after initiation of drug treatment.

Table 4. The amount of *M. leprae* antigen excreted in the urine from patients who previously had been prescribed DDS and were admitted for supervised DDS treatment to establish whether they had developed secondary DDS resistance

Patient No.	Number of weeks on supervised DDS	DDS effective clinically + falling MI	MFP results	Amounts of *M. leprae* antigen before and after initiation of supervised DDS treatment									
				before	3–4d*	1w	2w	3w	4w	8w	16w	32w	52w
98	4w	yes	—	470	410	240	200		800				
102	8w	yes	MR	55	180	440		750	310	100	< 20		< 20
106	4w	yes	—	24	23	< 20	< 20	< 20		< 20	< 20		
109	4w	yes	MR	35		170	360	115	88	26	82	< 20	
110	8w	yes	LR	34	40	90	36	32	250	180			
111	4w	no	MR	44	100	110	160						
112	8w	no	HR	670	810		1100	120		1700			

* See Table 3

Table 5. The amounts of *M. leprae* antigen excreted in the urine from patients given combined drug therapy after initial supervised DDS monotherapy

Patient No.	Drug treatment after initial DDS	Amounts of *M. leprae* antigen before and after initiation of combined drug treatment									
		before	3–4d*	1w	2w	3w	4w	8w	16w	32w	48w
98	DDS + Rif.(3w)	800	410	96	240	520	240	120	180		< 20
100	DDS + Rif.(3w)	< 20	< 20	< 20		210	110	< 20			
111	Rif. + Clof.(3w)	160	87	90	62	35	29			20	
112	Rif. + Clof.(3w)	1700	790	570		1900	950				
113	Rif. + Clof.(3w)	62	140	60	63	57	180	99	< 20	70	

* See Table 3

decreased over time. Two of them had peak excretion 3 and 4 weeks after initiation of this treatment.

Discussion

The present study indicates that in most of the lepromatous leprosy patients the decrease and disappearance of *M. leprae* antigen from urine, as measured by the presently used RIA, comes 2–4 months after start of effective treatment and coincides with the falling of MI[7,8] and clinical improvement in successfully treated cases. Big quantities of degenerated bacilli are present within the lepromatous lesions in patients with lepromatous leprosy for very long times, in spite of successful treatment, as seen in slit-skin smears with the presence of fragmented and granulated acid-fast bacilli for 4 years or longer. The antigen level appears therefore to fall at a much faster rate than the quantity of acid-fast material in the tissues. These findings suggest that the urine antigen as measured in the present RIA can be an early breakdown product of killed bacilli. Our conclusion is that the *M. leprae* antigen in urine seems to be another promising way to follow the early initial success or failure of treatment. But this needs to be confirmed in a larger number of patients, especially DDS-resistant cases followed for a longer period of time, after refinement of the assay before any firm conclusions can be drawn. Other antigens like PGL-I which has also been found in urine[9] should then also be included in the studies.

The amount of excreted urine *M. leprae* antigen after the start of effective drug treatment does not follow a constant course as assayed in the present study but some main patterns can be seen. In the most aggressively treated patient (No. 103), who received a combination of four drugs, a steady decline of antigen to negativity was seen in 8 weeks. In most of the other patients a peak of antigen excretion was seen after a few weeks of treatment. This peaking is, however, hard to evaluate due to the spread of excreted amounts of antigen as seen from the comparison between two different 24-hr periods before the start of or change of treatment. In this comparison less than a 5-fold difference was seen in 10 of the 13 patients, 5·5-fold in two and 7·5-fold in one. Several explanations of this variation can be given. It is for example a well-known fact that sampling of all the urine produced during a 24-hr period can be hard to accomplish. Another possibility is that some antigen was lost during the procedures of sampling and concentration. The variation may also reflect a true phenomenon, since before the *M. leprae* antigen reaches the sampling vessel for the urine, it must probably successfully pass a long chain of events: 1, to dissolve from the *M. leprae* bacillus in a form that is antigenic; 2, transport from the intracellular location to the extracellular space; 3, transport to the general circulation via the lymphatics and/or local venous routes; 4, to resist the specific and unspecific systems of clearing foreign material in the circulation which include immunocomplex formation. Antigens that are

poor immunogens therefore ought to be more likely to overcome this clearing; 5, excretion to the urine by glomerular filtration or tubular excretion; and 6, to avoid neutralization by anti-*M. leprae* antibodies prevalent in the urine.[10] Minor changes in one or several of these consecutive steps from day-to-day may explain the interday variations in *M. leprae* urine antigen excretion. A direct deposition of *M. leprae* antigen in urine from bacilli close to the urinary tract can however not be excluded even though bacilli were not found in the urine of Ethiopian leprosy patients in an earlier study.[2]

The changes of urine-antigen excretion during effective treatment were significant by the finding that eight out of the 18 patients were followed to antigen negativity (≤ 20 μg/24 hr). In eight of the 10 patients whose last sample was positive, changes of antigen amount were ≥ 8-fold while it stayed in the same range of variation as the control-samples for two of the 10.

Antigen assay of urine is an interesting possibility in deciding if a patient can stop multidrug therapy for lepromatous leprosy or not. However, four of eight patients treated with DDS monotherapy turned negative 1–32 weeks after start of treatment, a period obviously much too short for treatment with DDS as monotherapy in these patients.

In order to evaluate further the possible applications for antigen detection and quantification in the urine, the antigens to be found in urine should be characterized biochemically and immunologically and work is in progress along these lines.

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