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On the Etiology of Cat Leprosy[1]

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"Cat leprosy" was first described by Brown *et al* (1). They concluded that the clinical and histopathologic features suggest that the disease resembles both human and rat leprosy. Lawrence and Wickham (3) stated that the ability to produce typical experimental rat leprosy on the one hand and the failure of the bacillus to grow on artificial media on the other strongly suggest that it is in fact *Mycobacterium lepraemurium*. More recently, D'Arcy Hart and Rees (2) concluded from studies on the elongation *in vitro* of mycobacteria that a strain of cat leprosy appeared to be *M. lepraemurium* rather than *M. leprae*. No further evidence in support of this hypothesis has been reported. The criteria mentioned above cannot be regarded as conclusive evidence that the bacillus from cat leprosy and *M. lepraemurium* are identical.

The present study was set up to obtain immunological evidence in favor or against the hypothesis of identical strains. Two groups of guinea pigs were sensitized to mycobacteria from cat leprosy and to *M. lepraemurium*, respectively. The delayed-type hypersensitivity skin reactions to lepromins made from both strains were compared. If both strains are identical, a similar pattern of reaction can be expected. If both strains are not identical, stronger reactions to the homologous antigen are to be expected. The experiments were made with mycobacteria from cat leprosy strain D70/480 (4).

MATERIALS AND METHODS

Preparation of suspensions for sensitization. Parts of cutaneous bacilliferous nodules from mice were homogenized in 0.1% albumin solution. The homogenate was centrifuged at 1,500 RPM for five minutes. The supernatant was centrifuged again for five minutes at 3,000 RPM. The supernatant thus obtained was discarded. The bacilliferous sediment was suspended in 0.1% albumin solution, standardized and used for sensitization.

Preparation of lepromin. Parts of bacilliferous skin nodules from mice were autoclaved. The epidermis and the subcutaneous fat were removed. The remaining tissue was minced and homogenized in 9 ml 0.5% phenol saline solution per gram of tissue. The suspension was allowed to sediment for 20 minutes after which the supernatant was collected and stored. The sediment was shaken firmly for ten seconds and then was left to sediment for twenty minutes. The supernatant was then added to the first supernatant. This procedure was repeated twice. Phenol saline solution was added to the reinforced supernatant until the original volume was obtained. In order to remove as many tissue components as possible, this whole procedure was repeated twice. The final lepromin was diluted until such a bacterial count was reached, which gave skin reactions which could be easily read.

First series. Two groups of six nine week old guinea pigs were sensitized by the intraperitoneal route with 0.2 ml suspension of mycobacteria from cat leprosy and *M. lepraemurium* respectively, containing 225×10^6 bacilli. Two groups each of two guinea pigs were not sensitized. Two months later intracutaneous skin tests were carried out with two lots of lepromins of different strength, made of mycobacteria from cat leprosy and *M. lepraemurium*. The test doses were 0.1 ml, containing 160×10^6 (series 1A) and 480×10^6 (series 1B) bacilli, respectively. All tests were carried out simultaneously.

Second series. The skin tests were repeated in a new series of guinea pigs with newly prepared sensitizing suspensions and lepromins, containing 225×10^6 and 160×10^6 bacilli per 0.1 ml, respectively.

RESULTS

Guinea pigs sensitized to mycobacteria from cat leprosy and *M. lepraemurium* developed visible and palpable skin reactions at the site of intracutaneous injection to antigens of both strains. After 24 hours a mild,

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TABLE 1. *Reactions of sensitized guinea pigs to lepromins of mycobacteria from cat leprosy and* M. lepraemurium.

Animal sensitization		Sensitization: mycobacteria from cat leprosy												Sensitization: *M. lepraemurium*											
Test antigen		Mycobacteria from cat leprosy						*M. lepraemurium*						Mycobacteria from cat leprosy						*M. lepraemurium*					
Time of reading in days[a]		2	7	10	14	18	21	2	7	10	14	18	21	2	7	10	14	18	21	2	7	10	14	18	21
Series 1A	1	0		3.5		3		3		5.5		4		3		4		4.5		4		5		5.5	
	2	3		4		3		3		6		4		0		5		5		4		6		6	
	3	3		3		4		3		6		4		0		0		0		0		2		4	
	4	3		5		4		6		6		4.5		4		5		4		4		5.5		6	
	5	0		3.5		4		4		5		4.5		0		5		5.5		4		7		5	
	6	0		4		3		3		6		5		4		3		4		5		5		5	
Average		1.3		3.8		3.7		3.8		5.8		4.3		1.8		3.6		3.8		3.5		5.1		5.2	
Series 1B	1	0		3		3		4		6		5		5		4		5		6		6		6	
	2	6		4.5		3		6		6		5		4		5.5		5.5		5		8		6	
	3	6		4		3.5		6		6		5		3		2		0		4		3		5	
	4	5		5		4.5		8		8		6.5		7		8.5		5		8		8		6.5	
	5	4		5		5		8		7		8.5		8		6		6		9		8		7	
	6	3		4.5		5		7		8		5		5		4		5		5		6		4	
Average		4		4.3		4		6.5		6.8		5.8		5.3		4.7		4.4		6.2		6.5		5.8	
Series 2	1	4	4	5	5		4	4	7	6	5		3	2	6	6	5		2	3	8	8	7		5
	2	3	6	6	6		4	3	6	8	7		5	4	5	5	5		3	4	7	7	7		5
	3	4	6	6	5		5	4	7	7	6		5	4	5	5	4		4	3	8	9	7		4
	4	3	5	5	4		4	3	6	7	7		5	4	6	5	5		3	4	9	9	8		4
	5	4	6	6	6		4	4	8	10	8		5	4	6	6	5		4	3	8	8	7		4
	6	4	7	7	6		4	3	10	9	6		4	3	6	6	5		4	3	9	8	7		4
Average		3.7	6.0	5.8	5.3		4.2	3.5	7.3	7.8	6.5		4.5	3.5	5.7	5.5	4.9		3.3	3.3	8.2	8.2	7.2		4.3

[a]All readings in millimeter diameters of reactions.

TABLE 2. *Reactions to lepromins of mycobacteria from cat leprosy and* M. lepraemurium *in nonsensitized controls.*

Test antigen	Mycobacteria from cat leprosy							*M. lepraemurium*						
Time of reading in days[a]	1	2	7	10	14	18	21	1	2	7	10	14	18	21
Series 1A	0	0		0		0		0	0		0		0	
	0	0		0		0		0	0		0		3.5	
Average	0	0		0		0		0	0		0		1.5	
Series 1B	0	0		0		0		0	0		1.5		3	
	0	0		1		3.5		0	0		1.5		0	
Average	0	0		0.5		1.5		0	0		1.5		1.5	
Series 2	3	2	2	0	0		0	4	2	2	0	0		0
	0	0	0	0	0		0	0	0	0	0	0		0
Average	1.5	1	1	0	0		0	2	1	1	0	0		0

[a]All readings in millimeter diameters of reactions.

erythematous, early reaction was seen. This early reaction subsided in the next 24 hours.

After two days a papular lesion developed. This late reaction increased in size during the following week reaching a maximal size at an average of ten days after injection of the lepromin (Table 1). In nonsensitized guinea pigs reactions to the lepromins were absent or negligible (Table 2).

Figure 1 shows that the pattern of the reactions in all test series was identical. Antigens with a higher bacterial count produce stronger reactions in the same animals as compared with weaker antigens. In all the series the reactions to murine lepromin were on the average stronger than to lepromin of mycobacteria from cat leprosy. Because the difference was observed in all series it can-

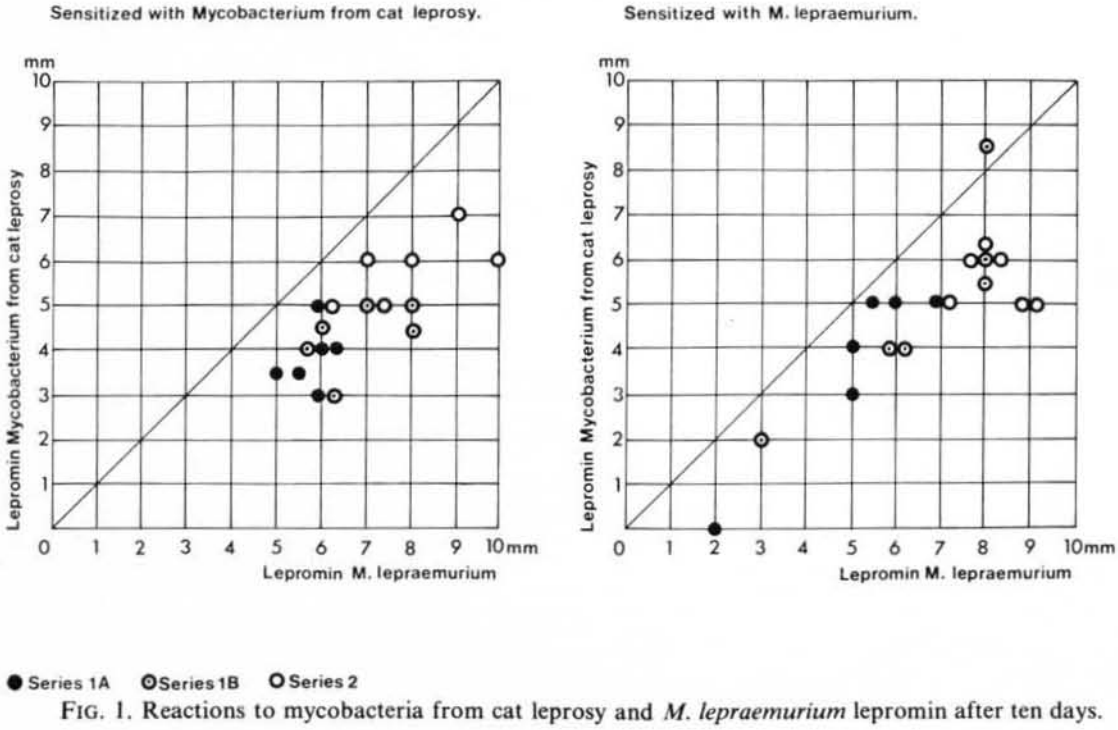

FIG. 1. Reactions to mycobacteria from cat leprosy and *M. lepraemurium* lepromin after ten days.

not be caused by technical errors in standardization, but may be due to a greater immunological activity on the part of the murine strain.

The identical pattern of reactions to *M. lepraemurium* and mycobacteria from cat leprosy lepromins in all series and the absence of stronger reactions to homologous antigens as compared to heterologous antigens is regarded as evidence that the mycobacteria from the cat leprosy and *M. lepraemurium* are identical.

SUMMARY

Two series of guinea pigs were sensitized with standardized suspensions of living mycobacteria from cat leprosy and *M. lepraemurium* respectively. After two months, simultaneous intracutaneous skin tests were carried out with standardized lepromins made of both strains. Delayed-type hypersensitivity skin reactions to both lepromins, reaching a maximal size after about ten days, occurred in all animals. In nonsensitized animals reactions were absent or negligible. The pattern of reactions to both antigens was found to be identical in all series. Because homologous antigens did not produce stronger reactions than heterologous antigens, it is concluded that *M. lepraemurium* and mycobacteria from cat leprosy are identical.

RESUMEN

Se sensibilizaron dos grupos de cobayos con suspensiones estandardizadas de micobacterias de lepra del gato y *M. lepraemurium* respectivamente. Después de dos meses se efectuaron pruebas intradérmicas simultáneas con leprominas estandardizadas preparadas con ambas cepas. En todos los animales se observaron reacciones cutáneas del tipo de hipersensibilidad tardía con ambas leprominas, las cuales alcanzaron un tamaño máximo después de alrededor de 10 días. En animales no sensibilizados las reacciones no se produjeron o fueron insignificantes. Se encontró que el tipo de reacción a ambos antígenos fué idéntico en todos los casos. Ya que los antígenos homólogos no produjeron reacciones mayores que los antígenos heterólogos, se concluye que el *M. lepraemurium* y la micobacteria de la lepra del gato son idénticas.

RÉSUMÉ

Deux séries de cobayes ont été sensibilisés avec des suspensions standardisées, d'une part de mycobactéries vivantes isolées de lèpre du chat, et d'autre part de *M. lepraemurium*. Après deux mois, des épreuves intra-cutanées ont été menées simultanément avec des lépromines standardisées préparées à partir de ces deux souches. Des réactions cutanées d'hypersensibilité du type retardé a ces deux lépromines, ont été observées chez tous les animaux; ces réactions ont atteints leur dimension maximale après environ dix jours. Les animaux non sensibilisés n'ont pas présenté de réaction, ou seulement des réactions négligeables. L'aspect des réactions à l'un et l'autre de ces antigènes s'est révélé identique dans toutes les séries. Puisque les antigènes homologues n'entrainent pas de réactions plus prononcées que les antigènes hétérologues, on en conclut que *M. lepraemurium* et les mycobactéries de la lèpre du chat sont identiques.

REFERENCES

1. Brown, L. R., May, C. D. and Williams, S. E. A nontuberculous granuloma in cats. NZ Vet. J. **10** (1962) 7-9.
2. D'Arcy Hart, P. and Rees, R. J. W. Elongations *in vitro* of *Mycobacterium lepraemurium* as a distinction from *Mycobacterium leprae*. Int. J. Lepr. **36** (1968) 83-86.
3. Lawrence, W. E. and Wickham, N. Cat leprosy: infection by a bacillus resembling *Mycobacterium lepraemurium*. Aust. Vet. J. **39** (1963) 390-393.
4. Poelma, F. G. and Leiker, D. L. Cat leprosy in the Netherlands. Int. J. Lepr. **42** (1974) 307-311.