

Vet. Med. J., Giza, 39, No. 1, 29-45 (1991)

**DELAYED HYPERSENSITIVITY AND HUMORAL
IMMUNE RESPONSE IN STEERS EXPERIMENTALLY
INFECTED WITH DIFFERENT STRAINS
OF MYCOBACTERIA**

BY

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(Received: 27. 1. 1991)

INTRODUCTION

Animals infected with mycobacteria are allergic to the protein contained in PPD tuberculin and develop characteristic delayed - type hypersensitivity reactions when exposed to tuberculoproteins in tuberculin. If tuberculin is deposited in the skin (intradermally), a local reaction characterized by inflammation and swelling usually is elicited in infected animals, whereas healthy animals fail to develop such responses at the injection site (Thoen, 1988). The comparative tuberculin test in which two or more tuberculins are injected simultaneously offers advantages in differentiating infection with *M. bovis* from various non-specific infections (Francis et al., 1978). The tuberculin positive cattle show also changes in the serum protein constituents (Moses et al., 1975 and Gatner et al., 1982).

The aim of this study was to followup the delayed hypersensitivity responses in steers infected with a variety of mycobacterial species and the changes in the immune response determined by immunoglobulin levels in their sera.

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MATERIALS AND METHODS

Animals:

Five steers were used in this study. These steers were tuberculin tested one week before experimental infection and showed negative tuberculin reaction. The first four animals were individually inoculated with an infective dose of only one of any of *M. bovis*, *M. tuberculosis*, *M. kansasii* and *M. fortuitum* according to Wedman et al., (1964). The fifth steer was left as control.

Tuberculin Test:

All steers were tested by the single comparative intradermal tuberculin test according to Lesslie and Hebert (1975) using the human, bovine and avian tuberculins. The interpretation of the test was done according to Anon (1963 and 1977).

Quantitative Determination of Total Serum Proteins and its Fractions:

Serum proteins were determined by the method of Cannon et al., (1974). Electrophoresis and densitometric determination of serum protein fractions were done according to Smith (1969).

RESULTS

Tables 1,2,3, and 4, showed the results of the single intradermal comparative tuberculin tests in *M. bovis*, *M. tuberculosis*, *M. kansasii* and *M. fortuitum* experimentally infected steers respectively. The non inoculated control steer showed negative tuberculin reactions during the whole experimentation period.

Tables 5,6,7, and 8 demonstrated the details of changes in serum protein fractions at different intervals after experimental infection with different strains of Mycobacteria.

Table (1): Comparison between single intradermal comparative tuberculin test using bovine/avian PPD (B/A S.I.D.C) and human / avian PPD (H/A S.I.D.C) in M. bovis experimentally infected steer.

WEEKS POST INFECTION	Increase in skin thickness(mm)			B - A	H - A	Interpretation	
	Bovine PPD	Human PPD	Avian PPD			B/A	H/A
				S.I.D.C	S.I.D.C		
A	0	0	0	0	0	-ve	-ve
2	1	2	2	-1	0	-ve	-ve
4	40	20	7	33	13	+ve	+ve
6	38	27	3	35	24	+ve	+ve
8	37	17	3	34	34	+ve	+ve
10	37	15	2	35	13	+ve	+ve
12	36	16	2	34	14	+ve	+ve
14	36	16	3	33	13	+ve	+ve
16	33	15	3	30	12	+ve	+ve
18	32	14	3	29	11	+ve	+ve
20	31	13	3	28	10	+ve	+ve
22	30	13	3	27	10	+ve	+ve
24	26	12	3	23	9	+ve	+ve
26	22	11	2	20	9	+ve	+ve
28	20	10	2	18	8	+ve	+ve

A = one week before experimental infection.

B-A = difference between bovine and avian reaction.

H-A = difference between human and avian reaction.

Table (2): Comparison between single intradermal comparative tuberculin test using bovine / avian PPD (B / A S.I.D.C) and human / avian PPD (H/A S.I.D.C) in M. tuberculosis experimentally infected steer.

Weeks post infection	Increase in skin thickness (mm)			H - A	H - A	Interpretation	
	Dovine PPD	Human PPD	Avian PPD			B/A S.I.D.C	H/A S.I.D.C
A	0	0	0	0	0	-ve	+ve
2	1	1	2	-1	-1	-ve	+ve
4	15	21	8	7	13	+ve	+ve
6	28	36	6	22	30	+ve	+ve
8	26	23	5	21	18	+ve	+ve
10	24	22	5	19	17	+ve	+ve
12	23	21	4	19	17	+ve	+ve
14	22	22	4	18	18	+ve	+ve
16	21	22	4	17	18	+ve	+ve
18	20	20	4	16	16	+ve	+ve
20	19	17	4	15	13	+ve	+ve
22	17	16	4	13	12	+ve	+ve
24	16	15	4	12	11	+ve	+ve
26	15	15	4	11	11	+ve	+ve
28	13	14	3	10	11	+ve	+ve

A= one week before experimental infection.

B-A= difference between bovine and avian reaction.

H-A= difference between human and avian reaction.

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DISCUSSION

The five steers were tuberculin tested a week before the experimental infection and then were tested every 2 weeks (Moodie, 1977; Awad et al., 1979; and Thoen et al., 1983 a) until the 28th week by the single comparative tuberculin test using bovine / avian and human / avian PPDs. Interpretation of the tests was done according to Anon (1977) and Anon (1963) respectively. All animals proved to be tuberculin negative before experimental infection and until the 4th weeks post infection.

The steer infected with *M. bovis* (Table 1) showed a notably higher reaction to the homologous tuberculin than that of the human PPD tuberculin, while it was negative to the avian PPD tuberculin. These results agree with that of Lesslie et al., (1975), Lutsevich (1976) and Petricevic et al., (1977).

The steer infected with *M. tuberculosis* (Table 2) gave a higher reaction to the homologous PPD tuberculin than the bovine PPD tuberculin from the 4th to the 6th weeks after infection, and showed no significant difference between the bovine and human tuberculins from the 8th to the 28th weeks. This agrees with the findings of Francis et al., (1978). An unexpected positive reaction to the avian PPD tuberculin was shown in this animal although the mammalian readings were still the higher. It is difficult to interpret such a result in an animal showing a negative tuberculin reaction to any of the bovine, human and avian PPD tuberculins before starting artificial infection. It is known that *M. tuberculosis* infection does not elicit non-specific sensitization to avian PPD tuberculin; otherwise the animal may have been sensitized to avian TB or other atypical mycobacteria. It may be said that this particular steer may have been exposed by a way or another to some kind of sensitization week or so before starting the experiment.

Table (3): Comparison between single intradermal comparative tuberculin test using bovine / avian PPD (B / A S.I.D.C) and human / avian PPD (H/A S.I.D.C) in M: kansasii experimentally infected steer.

Weeks post infection	Increase in skin thickness(mm)			B- A	H - A	Interpretation	
	Bovine PPD	Human PPD	Avian PPD			B/A S.I.D.C	H/A S.I.D.C
A	0	0	0	0	0	-ve	-ve
2	1	1	4	-3	-3	-ve	-ve
4	1	2	2	-1	0	-ve	-ve
6	5	8	3	2	5	±	±
8	7	10	5	2	5	±	±
10	5	8	3	2	5	±	±
12	4	9	3	1	5	±	±
14	4	9	3	1	6	±	±
16	1	2	2	-1	0	-ve	-ve
18	2	2	5	-3	-3	-ve	-ve
20	1	2	2	-1	0	-ve	-ve
22	1	1	2	-1	-1	-ve	-ve
24	1	1	1	0	0	-ve	-ve
26	0	1	1	-1	0	-ve	-ve
28	0	0	0	0	0	-ve	-ve

A = one week before experimental infection.
 B-A = difference between bovine and avian reaction.
 H-A = difference between human and avian reaction.

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The steer infected with *M. kansasii* showed non-specific sensitization to bovine, human and avian PPD tuberculins starting from the 6th week post infection up to the 14th week, with maximal readings at the 8th week (Table 3). Sensitization to such an infection seems to wane later on from the 16th week until it abolished afterwards. According to Anon (1977) and Anon (1963), the interpretation of the bovine avian and human - avian comparative tuberculin test revealed an inconclusive reaction throughout that period when the animal showed non-specific sensitization i.e. 8 weeks.

The steer infected with *M. fortuitum* showed a weak non-specific sensitization to both bovine and avian PPD tuberculins, and a somewhat stronger non-specific sensitization to human PPD tuberculin starting from the 6th week to the 10th week post infection (Table 4). Sensitization to such an infection seems to wane later on from the 12th week until it disappeared by the end of the experiment. According to Anon (1977) and Anon (1963) the interpretation of the bovine - avian and human - avian comparative tuberculin test revealed also an inconclusive reaction throughout that period when the animal showed non-specific sensitization i.e. 4 weeks. These results coincide with that reported by Chaparas and Maloney (1978) who stated that *M. kansasii* has more common antigens than other mycobacteria. The non-specific reactions due to *M. kansasii* and *M. fortuitum* agree with the results of Korzh et al., (1978) who reported that *M. kansasii* and *M. fortuitum* gave non-specific reaction to mammalian PPD tuberculin. It is also noticed (Table 3 and 4) that atypical mycobacteria could sensitize animals to tuberculin test which decreased with time. These findings coincide with Schliesser (1965 and 1969); Lesslie et al., (1975), Thoen et al., (1977) and Corner and Pearson (1978). The *M. fortuitum* infected animal gave higher readings with the mammalian tuberculins than the avian tuberculin. This agrees with the findings of Thoen (1990).

Table (4): Comparison between single intradermal comparative tuberculin test using bovine/avian PPD (B/A S.I.D.C) and human/avian PPD (H/A S.I.D.C) in *M. fortuitum* experimentally infected steer.

Weeks post infection	Increase in skin thickness (mm)			B - A	H - A	Interpretation	
	Bovine PPD	Human PPD	Avian PPD			B/A S.I.D.C	H/A S.I.D.C
A	0	0	0	0	0	-ve	-ve
2	1	1	1	0	0	-ve	-ve
4	1	3	2	-1	1	-ve	±
6	5	8	3	2	5	±	±
8	5	9	3	2	6	±	±
10	4	8	3	1	5	±	±
12	2	2	3	-1	-1	-ve	-ve
14	1	2	2	-1	0	-ve	-ve
16	2	1	3	-1	-2	-ve	-ve
18	2	1	2	0	-1	-ve	-ve
20	1	1	2	-1	-1	-ve	-ve
22	0	1	2	-2	-1	-ve	-ve
24	0	0	1	-1	-1	-ve	-ve
26	0	0	1	-1	-1	-ve	-ve
28	0	0	0	0	0	-ve	-ve

A = one week before experimental infection.

B-A = difference between bovine and avian reaction.

H-A = difference between human and avian reaction.

5) Electrophoretic pattern of total serum protein and protein fractions of *B. bovis* experimentally infected steer.

Infection	Total proteins	Alb. gm %	Globulins gm %			Total globulins gm %	A/G ratio
			alpha	beta	gamma		
A	10.83	4.72	1.04	1.39	3.69	6.12	0.77
1	8.50	3.68	0.94	1.06	2.81	4.81	0.76
2	12.00	4.92	1.24	1.60	4.24	7.08	0.69
3	7.50	3.19	0.68	0.99	2.63	4.31	0.74
4	12.00	4.72	1.27	1.65	4.36	7.28	0.65
5	14.17	5.38	1.48	2.00	5.31	8.78	0.61
6	10.00	3.83	1.06	1.40	3.34	6.17	0.62
7	9.17	3.58	0.95	1.26	3.71	5.55	0.65
8	8.00	2.95	0.75	1.17	3.10	5.03	0.59
9	11.67	3.67	1.45	1.79	4.75	8.00	0.46
10	11.50	3.40	1.40	1.88	4.99	8.27	0.41

A = one week before experimental infection.

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Concerning the electrophoretic analysis of serum proteins in experimentally infected steers, it is evident that there was a significant ($P < 0,05$) increase in the total globulins in *M. bovis* infected steers that reached 8.2 g % at 29 weeks post infection in comparison to its level before infection 6.12 g %. Consequently the albumin globulin ratio was decreased from 0.77 to 0.41 during the same period. These findings agree with the results of Volk et al., (1953), Reda et al., (1957) and Iliev et al., (1961). Meanwhile the electropherogram of the serum of the steer experimentally infected with *M. tuberculosis* revealed an increase in the total globulins after infection, reached 6.88 g% at 24 weeks after infection but it returned to the level before infection at 27 weeks. The explanation of this increase may be due to the fact that any stimulation of the reticuloendothelial system as occurs in case of infection results in hyperglobulinaemia, and also due to the great similarity between human and bovine tubercle bacilli (Wayne 1982). The return of total globulins to its level before infection could be explained by the arrest of the disease (Volk et al., 1953) or it became inactive (Karlson 1962) or may be due to the elevation of the animal resistance.

The infection with *M. kansasii* and *M. fortuitum* resulted in minor changes both in serum protein fractions and A/G ration in comparison to its level before infection, a finding which agrees with similar findings in other species of animals (Weimer et al., 1954).

The electrophoretic pattern of the control steer serum protein showed no significant alteration along the experiment.

Table (6): Electrophoretic pattern of total serum protein and protein fractions of M. tuberculosis experimentally infected steer.

Weeks post infection	Total proteins	Alb. gm %	Globulins gm %			Total globulin gm %	A / G ratio
			alpha	beta	gamma		
A	8.50	4.02	0.87	0.88	2.72	4.47	0.90
3	10.17	4.01	1.08	1.73	1.49	4.30	0.93
6	8.67	4.00	0.79	1.26	2.63	6.68	0.83
9	9.17	4.16	0.72	1.17	3.11	5.00	0.83
12	11.67	5.26	1.46	2.07	2.87	6.40	0.82
15	8.33	3.52	0.91	1.19	2.71	4.81	0.73
18	9.17	3.45	1.16	1.19	3.37	5.72	0.60
21	6.67	2.81	0.71	0.96	2.19	3.86	0.73
24	12.00	5.12	1.34	1.80	3.74	6.88	0.74
27	8.00	3.95	0.47	0.94	2.64	4.05	0.97
29	8.30	3.04	0.46	0.92	2.98	4.36	0.90

A = one week before experimental infection.

Table (7): Electrophoretic pattern of total serum proteins and protein fractions of M. kansasii experimentally infected steer.

Weeks post infection	Total proteins	Alb. gm %	Globulins gm %			Total globulins gm %	A / G Ratio
			alpha	beta	gamma		
A	9.17	4.71	1.22	0.88	2.34	4.45	1.06
3	10.83	6.52	0.23	1.12	2.96	4.31	1.51
6	6.33	2.87	0.55	0.78	2.07	3.40	0.84
9	8.67	4.08	0.63	1.08	2.87	4.58	0.89
12	6.33	3.72	0.34	0.62	1.65	2.61	1.42
15	10.33	5.54	0.93	1.06	2.80	4.79	1.16
18	8.50	4.40	0.78	0.87	2.45	4.10	1.07
21	9.17	3.91	1.21	1.00	3.06	5.27	0.74
24	10.83	5.62	0.90	1.18	3.13	5.21	1.08
27	13.33	7.32	0.81	1.42	3.79	6.00	1.22
29	12.23	6.64	0.90	1.28	3.40	4.59	1.41

A = one week before experimental infection.

Table (8) : Electrophoretic pattern of total serum protein and protein fractions of *M. fortuitum* experimentally infected steer.

Weeks post infection	Total proteins	Alb. gm %	Globulins gm %			Total globulins gm %	A /G ratio
			alpha	beta	gamma		
A	10.00	3.60	1.37	1.38	3.65	6.40	0.56
3	12.50	4.22	1.31	1.91	5.05	8.27	0.51
6	11.67	3.26	1.38	1.92	5.10	8.41	0.39
9	12.50	3.54	1.22	2.11	5.59	8.92	0.40
12	12.51	3.99	1.28	1.98	5.24	8.50	0.47
15	11.67	4.57	1.05	1.08	4.97	7.09	0.64
18	9.17	3.39	0.86	1.35	3.57	5.78	0.59
21	8.67	3.34	0.84	1.23	3.25	5.33	0.63
24	9.50	3.99	1.24	1.25	3.07	5.56	0.72
27	8.67	3.10	1.00	1.25	3.32	5.57	0.56
29	8.92	3.24	0.92	1.30	3.44	5.67	0.57

A = one week before experimental infection.

*Delayed hypersensitivity and.....***SUMMARY**

Four steers were inoculated with *M. bovis*, *M. tuberculosis*, *M. kansasii* and *M. fortuitum* respectively. A fifth steer was left as an uninoculated control. Results of tuberculin test indicated that reaction to homologous tuberculin was more specific than heterologous tuberculin. Non specific reaction to bovine and human PPD tuberculins was developed in steers injected with strains other than *M. bovis*.

Electrophoretic analysis revealed significant increase in total globulins in the serum of the steer experimentally infected with *M. bovis* in comparison with the control steer. Meanwhile a slight rise in total globulins was noted in *M. tuberculosis* infected steer but it returned to normal level at the end of the experiment. A non - significant change in total globulins was noticed in the sera of the other inoculated steers.

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