

AN EXPERIMENTAL STUDY OF GRANULOMAS IN BUFFALO-CALVES INDUCED BY BGG*

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Granulomatous inflammation as a distinct entity was recognised in the early 19th century (Long, 1965). In the last two decades a concerted effort has been made to study the various aspects of granulomas in experimental animals. Adams (1974, 1975) studied the development of granulomas with primary injection of BCG and *Mycobacterium tuberculosis* in guinea-pigs. Since no such studies have been reported in domestic animals, a study was undertaken in buffalo-calves to elucidate the sequential development of granulomas by injecting BCG.

Materials and Methods

Three clinically healthy male buffalo-calves aged about six months weighing around 55-60 kg were used for inducing experimental granulomas. Sites measuring 3 sq. cm. were prepared for surgery at seven places on neck, thorax, and flank region 10 cm apart and were injected intradermally with ten human doses of BCG vaccine** reconstituted in 0.1 ml of pyrogen free distilled water.

Animals were kept under observation and their temperatures noted daily. The size of developing lesions were measured and excised surgically under local anaesthesia at the end of 24 hours and thereafter at weekly intervals upto the end of six weeks. Tissues were fixed in buffered formalin (10%), softened in 5% phenol solution, dehydrated, cleared in cedarwood oil and impregnated with paraffin wax. Sections were cut and stained with Haematoxylin and Eosin in routine manner. Before the lesions were excised, total leucocyte, differential and T-cell counts were made. T-cells were demonstrated by using alpha Naphtyl-acetate esterase and SRBC rosetting techniques (Vikram Reddy *et al.* 1980).

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** Vaccine manufactured by the BCG vaccine laboratory, Guindy, Madras, India. Publication No. 210 of the Department of Pathology.

Results

General condition of the animals was uneventful and normal. No thermal reaction was noticed. There was an increase in skin thickness ranging from 1 to 2.49 mm. The maximum thickness was noted at the end of the 7th day and the increase was statistically significant ($P < 0.5$).

Haematological observations

An increase in the total leucocyte count was observed at the end of 24 hours after vaccination, which declined later. Absolute lymphocyte and T-cell counts showed an increase until 21 days. However, increase in absolute lymphocyte count was only statistically significant (Table I).

Histopathological observations

24 hours

Lesions were characterised by acute inflammation with swelling of dermal connective tissue and mild infiltration of polymorphs, monocytes, few RBC's and perivascular oedema.

7th day

Pronounced infiltration with macrophages was observed in perivascular spaces with some of them spilling over into surrounding connective tissue (Plate: Fig A). Majority of the cells had hyperchromatic nuclei and small amount of eosinophilic cytoplasm, while few had large vesicular nuclei with fine chromatin and pink foamy cytoplasm (Epithelioid cells).

14th day

The cellular reaction was more pronounced and extensive with heavy infiltration of mononuclear cells in the perivascular areas and in between the connective tissue fibres. These cells were pleomorphic viz (a) cells with vesicular nucleus, fine chromatin, nucleolus, and moderate amount of eosinophilic, foamy or vacuolated cytoplasm (epithelioid cells). (b) cells with hyperchromatic nucleus and scanty cytoplasm (c) fusiform cells with oblong nucleus. Epithelioid cells were predominant.

21 days

The cellular infiltration was much more prominent with occasional foreign body giant cell containing several irregularly distributed nuclei and abundant eosinophilic cytoplasm (Plate: Fig B).

28 days

The histological picture was similar but reaction was more extensive than that of earlier period. In addition young fibroblasts were also seen.

35th and 42nd days

A progressive resolution of lesion with greatly decreased cellular infiltration and increasing fibroblastic proliferation was observed (Plate Fig C & D). Hypertrophy and hyperplasia of vascular endothelium and the cells of tunica media were observed in 24 hours to 21 days old. Hydropic degeneration was also noticed (Plate Fig. A).

Discussion

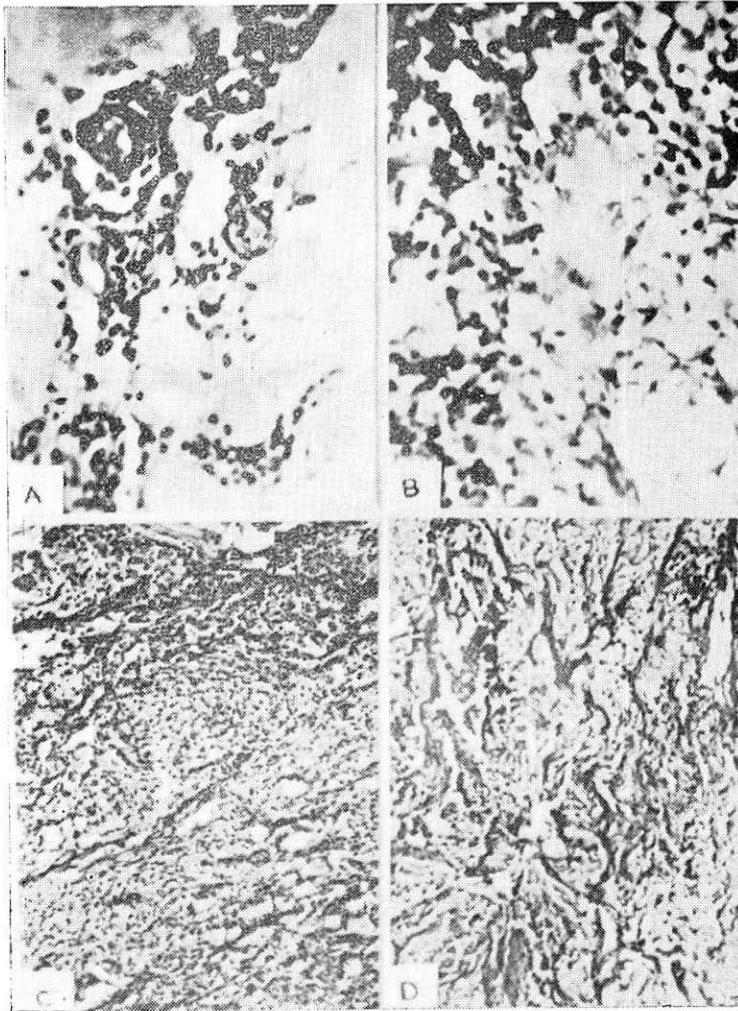
The initial acute inflammatory reaction and the development of the lesion into a mature and epithelioid granulomas around 7th and 14th day respectively were in broad agreement with similar studies made in guinea pigs by Adams (1974). However, foreign body giant cells were observed in the present study in contrast to Langhans giant cells observed by the above author. Lesions in the present study showed signs of resolution by 5th week and was complete in 6th week. Narayanan *et al.* (1981) however, observed resolution by the end of 7th week in guinea pigs. The above variation could be attributed to individual species response, dosage and strain of BCG used. Another feature noted was the development of lesions into epithelioid type of granuloma. In similar experimental studies, agents like BCG is known to induce "High turnover granuloma" of epithelioid type (Papadimitriou and Spector, 1972). In the present study induction of such a lesion in buffalo calves pointed towards a high turnover type of granuloma.

Table 1
Mean haematological values

Periods	Total leucocyte count	Lymphocyte count	T cell count
0 day	12166	6810 ^{bed}	1431
1 day	13233	9184 ^a	1768
7 day	12666	7855 ^{abe}	1716
14th day	11116	7245 ^{bc}	1645
21st day	12000	8055 ^{ab}	1632
28th day	9733	5373 ^d	1112
35th day	11166	5942 ^{cd}	1261
42nd day	10933	6009 ^{cd}	1179

Means having the same superscripts do not differ significantly ($P < .05$).

0 day = Values obtained 24 hours before BCG was administered to the animals.



- Fig. A. Experimental granuloma—BCG—7th day—perivascular oedema and infiltration of mononuclear phagocytes, proliferation of vascular endothelium with hydropic degeneration. HE x 100
- Fig. B. Experimental granuloma—BCG—21st day—heavy infiltration of macrophages, epithelioid cells and foreign body giant cells. HE x 320
- Fig. C. Experimental granuloma—BCG—28th day—extensive infiltration of macrophages, and epithelioid cells HEx20
- Fig. D. Experimental granuloma—BCG—35th day—Early signs of resolution—Cellular infiltration has greatly hinned out with increase in fibroblasts. HE x 50

Haematological studies revealed significant rise of lymphocyte count until 21 days. Such a rise is a common feature following vaccination. Other changes were not statistically significant. Studies on this aspect concurrently with sequential development have not been reported earlier.

Summary

Histopathological studies of granulomas induced by BCG vaccine experimentally were made in buffalo calves at different intervals. The important histopathological features were acute inflammatory reaction, development of mature and epithelioid granulomas at the end of 24 hours, 7th and 14th day respectively. Foreign body giant cells were seen in 21 day old lesions and thereafter. Hyperplasia, hypertrophy and hydropic degeneration were noticed in vascular endothelium and tunica media. Lesions showed evidence of resolution at the end of 35th day.

Concurrent haematological studies showed significant rise of lymphocytes till 21 days.

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