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BCG-induced Changes in the Post Capillary Venules of the Guinea Pig Lymph Node

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Summary

The post capillary venules (PCV) of the regional lymph nodes of BCG-injected male and female guinea pigs were examined under light and electron microscopy. The endothelial cells showed increased height and pyroninophilia. Their cytoplasm contained increased numbers of ribosomes and polyribosomes and prominent rough endoplasmic reticulum with occasionally dilated cisternae. The number of lymphocytes migrating through the PCV wall was also increased. The activation of the endothelial cells of PCV induced by BCG may increase the recirculation of lymphocytes from the blood stream. These may be of importance in the antitumour activity of BCG. In control guinea pigs the height of the endothelial cells of PCV was significantly greater in the female than in the male. After BCG injection this sex difference was still apparent.

Introduction

Previous studies have shown that the microcirculation of lymph nodes is changed under antigen-stimulation (1, 2). There is a recirculation of lymphocytes from the blood into the lymphatic tissues through the high endothelial-post capillary venules (PCV) of the lymph nodes (3). It is also found that the PCV undergo morphological change under various antigenic stimuli (4, 5, 6).

Hillman et al. (7), described microvascular alterations in lymph nodes responding to BCG inoculation which may be of importance in BCG-induced anti-neoplastic activity. No comment was made on morphological changes in the PCV. The inhibition of tumor growth using BCG is a combination of specific and non-specific immune mechanisms (7, 8). Since the lymphocytes entering the lymph nodes through the PCV are known to be necessary for the immune response (1, 9) an investigation was designed to determine the changes induced on the morphology of the PCV and the passage of lymphocytes through their wall, following inoculation of guinea pigs with BCG.

Materials and Methods

Twelve mature male and twelve female guinea pigs were used. Half of each group were given an intramuscular injection of 0.1 ml standard strength BCG (Glaxo Laboratories Ltd, Greenford, Middlesex, England) in the right vastus muscle, the remainder being left uninjected. For tuberculin testing, an intradermal injection of 0.1 ml of solution containing 10 units of PPD (Evans Medical Ltd., Liverpool, England) was given five weeks following BCG inoculation in a shaved area on the right flank, 4 cm in diameter. The reaction was read at 24, 48 and 72 hours and the erythema diameters were measured to the nearest mm. Lymph nodes from the right sciatic area were fixed in Carnoy's fluid. Sections were cut at 5 μm, processed to paraffin and stained with haematoxylin and eosin, Periodic acid Schiff (PAS) and methyl green-pyronin stains. Small fragments of each lymph node were fixed in 3 % phosphate-buffered glutaraldehyde (pH 7.4) for six hours at 4 °C, post-fixed in 1 % osmium tetroxide and then embedded in Araldite. Sections were stained with lead citrate

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and examined in a Philips EM 400 electron microscope.

Sections 1 μm thickness were stained with Toluidine blue and examined by light microscopy. The lymphocyte migration index was calculated, this being the ratio between the number of lymphocytes passing through the PCV wall and the number of their endothelial cells. From each lymph node 100 cells lying inside the basal lamina of the PCV were counted. The height of the endothelial cells of PCV was also assessed with a graticule (E_{17} Graticules Ltd, England) by a method previously used (10). The significance of differences between mean values of groups was analyzed by Student's t-test.

Results

All animals inoculated with BCG reacted positively to intradermal challenge with PPD. A positive skin test was usually apparent within 24 hours after PPD injection. The mean value of the maximum diameters in mm and the Standard error of mean was 15.16 ± 0.48 for the male group and 16 ± 0.57 for the female. The difference was not statistically significant.

On paraffin sections numerous lymphocytes were seen to migrate through the PCV wall and the endothelial cells showed increased pyroninophilia after BCG injection.

In 1 μm thick sections, a sex difference was revealed in the height of the endothelial cells of PCV of the control guinea pigs (Table 1). The female controls had significantly higher endothelial cells than the male ($P < 0.05$). A significant increase in the height of these cells was seen in both sexes after BCG inoculation ($P < 0.001$). The female BCG-injected guinea pigs had significantly higher endothelial cells than the male injected ones ($P < 0.01$). The number of migrating lymphocytes through the PCV wall was also significantly increased (Table 1 and Fig. 1) in the BCG-stimulated male and female animals ($P < 0.001$ in both sexes).

Electron microscopic study of the lymph node PCV of control guinea pigs showed the general features which have been previously described in other animals (2, 3). However, in female but not in male control and BCG-injected guinea pigs, a few endothelial cells contained distinctive single membrane-bounded osmiophilic structures, consisting mostly of electron dense granular material interrupted by light zones (Fig. 2). These inclusions are under further investigation.

Following BCG injection the PCV of the lymph nodes of both sexes showed characteristic changes. The cytoplasm of the majority of the endothelial cells became darker, showing numerous free ribosomes and polyribo-

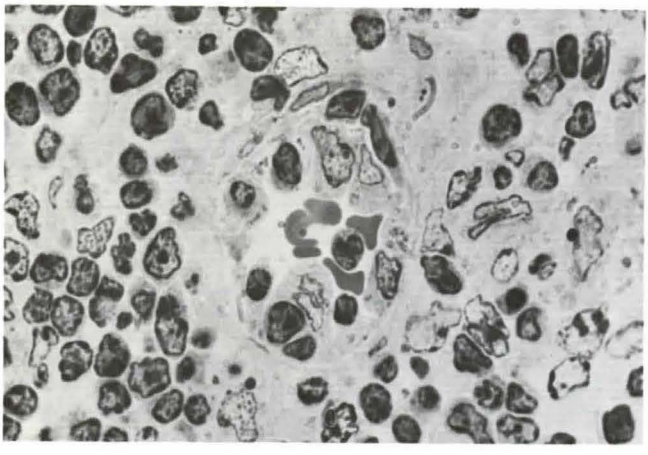

Fig. 1 Lymph node. Male BCG-injected guinea pig. Numerous lymphocytes migrate through the PCV wall. Toluidine blue x 700

Table 1 Influence of BCG inoculation on the number of lymphocytes migrating through the PCV wall and the height of the venular endothelial cells* *

Group	Lymphocyte Migration Index*		Height of endothelial cells of PCV (μm)	
	Male	Female	Male	Female
Control	0.88 ± 0.018	0.92 ± 0.022	9.1 ± 0.27	10.25 ± 0.33
BCG	1.26 ± 0.021	1.30 ± 0.024	12.26 ± 0.17	13.19 ± 0.21

*Lymphocyte migration index = number of migrating lymphocytes/number of endothelial cells of PCV.
**Results are expressed as mean ± Standard error of mean

somes and prominent rough endoplasmic reticulum with occasionally dilated cisternae (Fig. 2). The latter contained particulate material. Numerous mitochondria were found adjacent to rough endoplasmic reticulum. Golgi elements were present in some of the endothelial cells, but hey were absent from many others. Numerous lysosomes and phagolysosomes were also seen (Fig. 3). Lymphocytes were often seen attached to the luminal surface of the endothelial cells while clusters of migrating lymphocytes were present in intercellular spaces or trapped between the endothelial sheath and the pericytes (Fig. 4). No extravasation of red blood cells was seen.

Discussion

Inoculation with BCG induces the development of cell mediated immunity. However, the antitumor activity of BCG has also been studied increasingly over the past decade (8, 11, 12). Stimulated lymphocytes along with activated macrophages (8) and "natural killer cells" (13) have been considered as participating in the antineoplastic activity of BCG. *Hillman* et al. (7) showed that BCG induces hypervascularity in the guinea pig lymph node and suggested that this may play a part in the BCG-induced tumour immunosuppression.

The present study shows that in male and female guinea pigs there is a significant increase in the height of the PCV endothelial cells and in the number of lymphocytes migrating through the PCV wall of the lymph nodes draining the site of the BCG injection. The pyroninophilia, the increased numbers of ribosomes and polyribosomes, and the prominent rough endoplasmic reticulum with dilated cisternae seen in the endothelial cells after

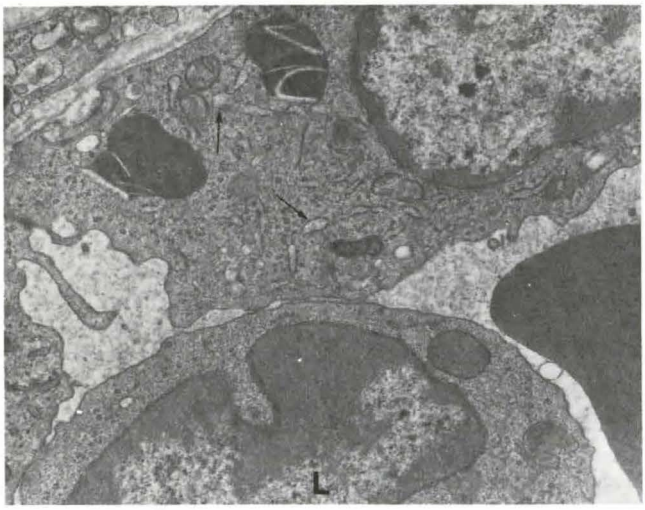

Fig. 2 Lymph node PCV. Female BCG-injected guinea pig. Numerous ribosomes and polyribosomes. Prominent rough endoplasmic reticulum with dilated cisternae (arrows). Two distinctive osmiophilic structures. L = lymphocyte. x 9050

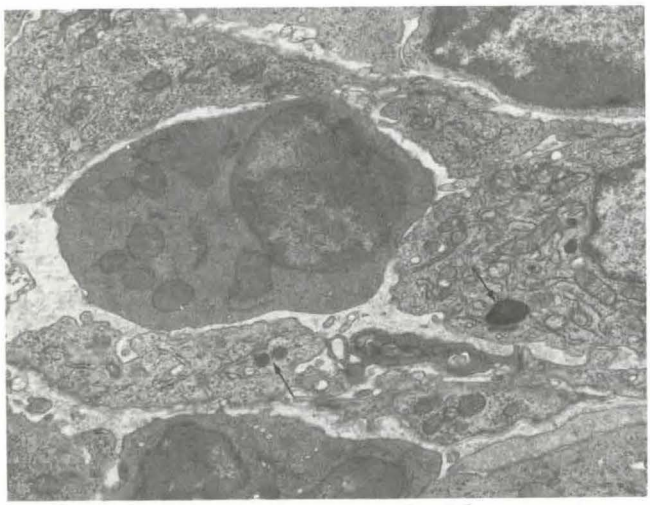

Fig. 3 Lymph node PCV. Male BCG-injected guinea pig. Numerous mitochondria adjacent to rough endoplasmic reticulum. Numerous ribosomes and several lysosomal elements (arrows). x 3.800

BCG inoculation represent evidence of increased protein production (14) while the numerous lysosomes and phagolysosomes are signs of phagocytosis. Similar changes involving the ultrastructural appearance and the height of the endothelial cells of PCV and also the number of lymphocytes crossing the PCV wall have been previously described in various species under a number of antigenic stimuli, but not BCG (1, 2, 4, 14).

The entry of lymphocytes from the blood into the lymph nodes is restricted to the PCV (15, 16). It is known that recirculating lymphocytes make an important contribution to the immune response (9). It is possible that the activation of the endothelial cells of PCV induced by BCG is related to the increase in the recirculation of lymphocytes which may be of importance in the development of delayed hypersensitivity and in antitumor activity of BCG.

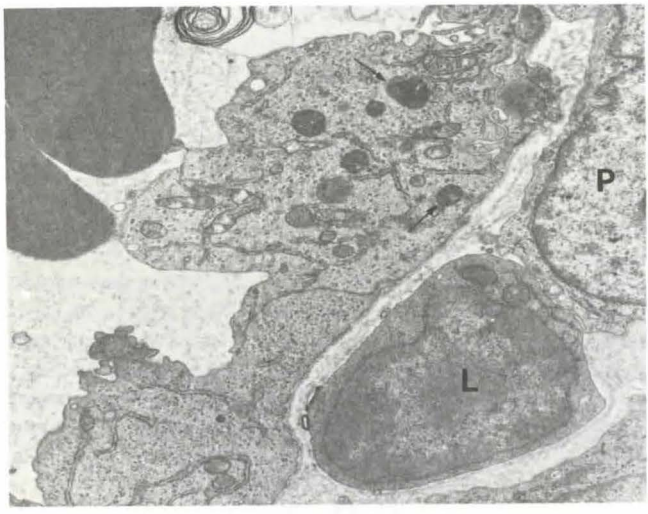

Fig. 4 As in Fig. 3. Numerous lysosomal elements (arrows). L = lymphocyte trapped between the endothelial sheath and a pericyte (P). x 4000

The present work also shows that there are significant sex differences in the height of the endothelial cells of PCV in the lymph nodes of male and female control guinea pigs. These differences were still apparent after BCG inoculation, in agreement with those seen in the mouse after injection with Toxoplasma gondii (5, 6, 14). It remains to be seen whether these observations are of significance in relation to the incidence of neoplasia in the two sexes as it has already been suggested that they are of significance in the incidence of autoimmune diseases (5, 6).

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