BCG ACTION UPON THE EVOLUTIVE RATE OF THE DISEASE SHOWN BY THE GOLDEN HAMSTER (CRISCETUS AURATUS) EXPERIMENTALLY INFECTED WITH MYCOBACTERIUM LEPRAEMURIUM*

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Many authors admit the induction of acquired resistance to the *M. leprae* infection, in man, due to BCG vaccination (11, 14, 16, 1, 27, 31, 30, 15, 4, 12, 33, 5, 6, 7, 9, 32, 8, 29, 10). However, this is a point to discuss because it has not solid bases.

The opinions about BCG action on the development of lesions which occur in rats, experimentaly infected with *M. lepraemurium*, are not in accordance. It was verified that previous BCG vaccination does not alter the intensity and the development of the lesions, (28, 20) or produces a partial inhibition in the evolutive rate of the lesions (2, 3, 23, 26). Yet, it has been settled that BCG administration does not modify the histological structure of the lesions produced by *M. lepraemurium* in rats (20); in other words, it does not modify the type of the host tissues reaction. The mycobacteria, either before or after BCG vaccination, are not lysed by the rat macrophage (21).

The inflammatory tissues reaction to *M. lepraemurium* in the rat is unchangeable and constitutes a property of this animal species (17, 18). It then appears, that this animal does not seem to be suitable for BCG action studies. The golden hamster, however, when inoculated with *M. lepraemurium* catches a disease which evolutes into two phases: a — initial phase, characterized by narrow and involutive lesions, which tend to spontaneous cure; b — final phase, verified after the 150th day of evolution, and which lesions evolute progressively up to the animal's death (22). The animals, apparently, present some resistance at the initial phase, which is not verified at the final phase. This finding led us to verify BCG action on the evolutive rate of the disease in hamsters inoculation by *M. lepraemurium*, since this animal presents a less stable tissues reaction than that presented by the rat.

MATERIAL AND METHODS

Golden hamsters of both sexes weighing 80-138 g, at the begining of the experience, were divided into two graups:

1 — 15 animals were inoculated subcutaneously with BCG previously killed by heating (water at 98°C for one hour). The BCG inviability was proved by culture. Each animal has received 20 mg of bacilli. At the 22nd day after BCG vaccination, these animals were infected intraperitoneally with 0.5 ml of a *M. lepraemurium* suspension, containing approximately 5 mg of bacilli. The dose of bacilli injected was determined by a method previously described (19).

2 — 14 animals, not previously vaccinated by BCG, were inoculated intraperitoneally with the same doses of *M. lepraemurium* (controls).

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Out of each group, only 3 animals were sacrificed. The other animals were left until natural death. The life span of each animal was recorded, constituting the basis for the survival study

The survival-time of the animals of these two groups has been statistically analysed. The basis for this analysis was the percentage of dead animals during the whole experiment.

The survival regression (% of dead animals on the time) for each group was then established. The comparison between the survival-regressive-lines in these two groups, was performed through co-variance analysis.

Histological studies were carried out upon the sacrificed and the naturally dead animals of these two groups. The material was fixed by Bouin's fluid or 10% formaline solution, embebedded in paraffin and stained with HE, ZiehlNeelsen, Masson's tricromic and Toluidine blue at pH 4.5. Histological studies were carried out during the several steps of the disease evolution and the histological aspect of the lesions was observed throughout the whole experiment (246 days).

RESULTS

The obtained results were based either on the survival-time or on macroscopic and microscopic aspects of the lesions.

The data concerning the survival-time shows differences between the control and BCG vaccinated group (Table I). In the first one, the mean of the survival-time is higher. The survival-time regressive-line shows a different sloping in the two groups (Figure), due to the different value of the regressive coefficients. The co-variance analysis of survival-time regressive-line in the two experimental groups (Table 2) reveals that these differences are significant. This data reveals that the survival in both groups differs significantly, and is higher at the control group.

Based on the survival-time, the BCG previous vaccination does not yield to the hamster a resistence to M. *lepraemurium* but, on the contrary, accelerates the rate of evolution of the experimentally induced disease, decreasing the survival-time. The shortening of the survival-time can be estimated by the regressive lines as being 59 days (around 25%).

The pathological study confirms the data obtained by the outliving analysis. It reveals that evolutive lesions appear earlier at the vaccinated group and evolute progressively, involving many organs. The macroscopic lesions are well established at around the 80th day of evolution on vaccinated animals and the control presents lesions of similar intensity only at about the 160th day.

At the initial period after the *M. lepraemurium* inoculation both groups (either the BCG vaccinated or the control) present a slight hypertrophy of mediastinic and cervical lymph nodes, slight splenomegaly and few peritoneal lesions. After 150 days in the control animals, and after 80 days in the vaccinated ones, the lesions have a larger size and involve, mainly, the lymph nodes and spleen, producing variable degrees of hypertrophy.

The differences between control and vaccinated groups are more outstanding from the histological aspect of the lesions.

The control animals present lesions chiefly in the spleen, but also in lymph nodes, omentum, liver and lungs. Initially they have a perivascular situation, arising from the resting connective tissue cells. As far as histological characters and number of bacilli are concerned, the lesions can be classified in two types (17): a) early or initial lesions; b) late lesions.

The early lesions are observed in control animals till the 6th — 7th month of the disease evolution. These animals have a little number only of bacilli, which generally show morfological alterations (fragmentation, aar granulations). The cells which take place in the inflammatory reaction, mainly the macrophages, are numerous when compared to the small number of bacilli and take a nodular arrangement; there are evidences that mycobacteria are lysed by the macrophage.

These lesions are morphologically similar to those presented by rats inoculated with heat killed bacilli; this means that these lesions have an involutive character. They are involved by lymphoid cells, with many plasmocytes. The lesion involution progresses, and at the 60th day after the inoculation the involutive aspect is very clear. By this time the lesions contain almost only plasmocytoid cells, which are grouped in nodules; often, giant cells can be seen among them. The plasmocytoid cells have some characters of the epitheliold cell. The early lesions tend to spontaneous cure, which occurs around the 180th day and the cicatrization is performed by fibrosis and hyalinization (22).

While the initial phase elapses, some macrophages can be seen, either alone or in small groups, containing in their cytoplasm numerous morphologically normal bacilli. This fact led us to suppose a continous bacillary dissemination, which is responsible for the appearing of late lesions and for the progressive development of the disease (22).

Late lesions appear in the control animals after the 150th day of evolution. They arise from connective perivascular cells not presenting a nodular shape in the begining; lately a nodular arrangement takes place. The lesion cells contain a great number of morphologically normal bacilli; the macrophage in this type of lesion does not lyse phagocytised mycobacteria, and for this reason it is transformed into the hamster leprous cell. The leprous cell may assume a plasmocytoid aspect (plasmocytoid leprous cell) or show a poliedric shape (poliedric leprous cell). Late lesions do not tend to necrosis and are not involved by lymphoid cells; their development rate is progressive and rapid.

The BCG vaccinated animals also present lesions that assume either the aspect of the initial or of the final lesions. The morphological aspect and cytogenesis of these lesions are identical to those seen in the lesions of the control animals. In the BCG vaccinated group, however, the early lesions are seen only until the 70th day of the evolution of the disease and they present a number of bacilli always higher than similar lesions in the control animals. These lesions do not tend to spontaneous cure, because they are precociously followed by final lesions, which alter their normal evolution. The initial and the final phases of the disease evolution are hardly identified in the BCG vaccinated animals. In these animals since the 30th — 40th day we can observe, at the same time, lesions with the morphological characters of both, the early and the final lesions. The early lesions are predominant. After the 70th day, almost every lesion has the microscopic structure of the late lesions, showing a rapid and progressive evolutive rate.

The evolutive rate of late lesions, however, appears to be similar, either in the vaccinated or in the control animals. This suggests that BCG vaccination abbreviates only the initial period of the disease.

The BCG vaccinated animals present, in many organs, a less intense hyaline substance deposition than the controls.

DISCUSSION

The experimental inoculation of *M. lepraemurium* produces in both, vaccinated and not vaccinated hamsters, a disease that evolutes to death. In the vaccinated animals, however, the disease presents a faster evolutive rate, because in these instances, the initial phase is shorter. There are evidences that in the initial phase, when the lesions are paucibacillary and show involutive aspect, the animal presents some degree of resistance. The resistance seems to depend upon the lysis of bacilli phagocytized by macrophages (22). Though bacillary lysis is slow, we can settle a correlation between its ocurrence and the morphological aspect of the early lesions; however, the existence of other factors, capable to intervene with M. lepraemurium growth in hamster tissues, cannot be forgotten. The active participation of hamster macrophages in the mycobacteria lysis, verified in early lesions, has permitted to place these cells between the rat

macrophage and the guinea-pig one (22). The rat macrophage is not able to lyse the M. lepraemurium (17), even when the animal is previously submitted to BCG vaccination (20, 21). On the contrary, the guinea-pig macrophage is able to lyse this mycobacterium even in the absence of BCG vaccination (17) and this ability is higher when such a vaccination is caried out (18). In these two animal species, BCG administration would not induce lytic properties to the macrophage against this mycobacteria since this cell, normaly, is not able of doing it; however, the BCG vaccination would increase this lytic property of the macrophages when these cells are already able to produce the mycobacterial lysis before vaccination.

Once hamster macrophages seem to be placed in an intermediary position between the rat guinea-pig macrophages, on the assertion that they are initially able to carry out mycobacterial lysis (early lesions), it could be supposed that BCG vaccination would intensify this activity. However, this does not occur. What really happens is an activation of the M. lepraemurium growth within the macrophages of the vaccinated hamsters. This activation is related to the faster appearing of late lesions. Apparently the previous administration of BCG stimulates the mycobacterium growth in hamster tissues.

The survival-time has been higher in control than in vaccinated hamsters. This is not a totally discordant data. When mice are vaccinated with more than 0.3 mg of BCG and then are infected with a virulent sample of M. tuberculosis there is also a decrease in the survival-time, if compared to non-vaccinated controls (13).

The usage of killed BCG is necessary for the hamster. This animal shows a special susceptibility to this bacillus, presenting a progressive and mortal disease when inoculated by alive BCG (24, 25). However, at least some antigenic properties of this bacillus must have changed with heat treatment.

SUMMARY

The influence of BCG vaccination upon the evolutive rate of the disease produced at -the golden hamster by M. lepraemurium inoculation was studied.

Fifteen hamsters were injected, subcutaneously, with 20 mg of BCG (killed by heating) and after 22 days were inoculated with 5 mg of M. lepraemurium, (controls).

The survival-time and the pathology of the lesions in these two animal groups have been studied.

The regressive lines of the survival-time (% of dead animals on the time) reveal significant differences between these two groups of animals. These differences are due to a higher survival-time found in non-vaccinated animals. Previous BCG vaccination turns faster the evolutive rate of M. lepraemurium experimental infection, in the hamster.

The histological study of the lesions reveals that the initial phase of the disease, which lesions have an involutive aspect, is shorter in the vaccinated group. As a consequence, late lesions are seen precociously and the development of the disease is faster. The evolutive rate of the late lesions does not seem to be modified by BCG administration.

There are evidences that in the early lesions of control hamster the macrophage is able to lyse the M. lepraemurium, although this lysis is slow.

These lytic properties of the macrophage are not activated by BCG vaccination, in opposition to what occurs with the guinea pig macrophages.

RESUMO

Foi estudada a influência da vacinação com o BCG sabre a evolução da moléstia produzida em "Hamster" (Criscetus auratus) pela inoculação de M. lepraemurium.

15 "Hamsters" foram injetados por via subcutânea com 20 mg de BCG (morto pelo aquecimento) e após 22 dias foram inoculados por via intraperitonial com, aproximadamente 5 mg de *M. lepraenmrium.* 14 outros animais foram injetados com a mesma dose de *M. lepraemurium*, (contrôles).

O tempo de sobrevida e as lesões patológicas dos 2 grupos de animais foram estudados.

A linha de regressão e o tempo de sobrevida (% de animais mortos em relação ao tempo) revelou diferença significante nos 2 grupos de animais.

Estas diferenças são devidas a um tempo de sobrevida mais prolongado, que se observa nos animais não vacinados. A vacinação prévia com o BCG acelera o ritmo evolutivo da infecção experimental pelo *M. lepraemurium* no "Hamster".

O estudo histológico das lesões revela que, a fase inicial da moléstia, cujas lesões possuem um aspecto involutivo, é mais curta no grupo dos animais vacinados. Em conseqüência, lesões tardias são observadas precocemente e o desenvolvimento da moléstia é mais rápido. O ritmo evolutivo das lesões tardias não é aparentemente modificado pela administração do BCG.

É evidente que, nas lesões iniciais nos animais contrôles o macrófago é capaz de lisar o *M. leproenturium* apesar desta lise ser lenta.

As propriedades líticas do macrófago não são ativadas pela vacinação com o BCG, ao contrário do que ocorre com os macrófagos do cobaio.

TABLE I

SURVIVAL TIME (IN DAYS) IN VACCINATED AND NOT VACCINATED GROUPS

Controls	BCG vaccinated
60	48
71	70
90	80
107	95
130	98
147	106
180	125
190	135
210	147
223	175
246	178
	183
MÉAN 150.4	120.0

TABLE II

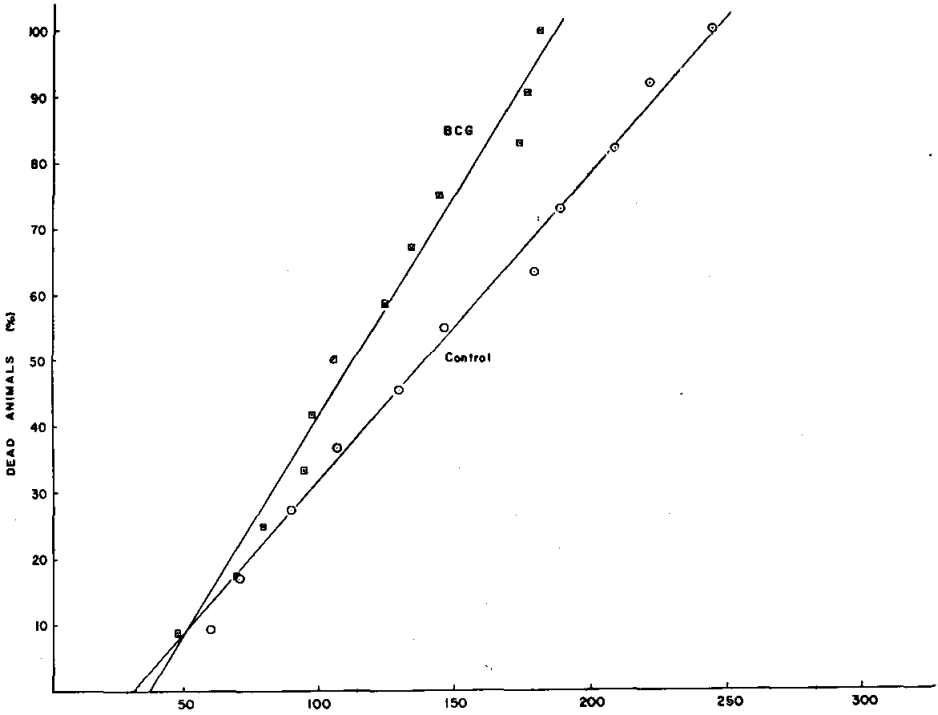
COVARIANCE ANALYSIS OF THE SURVIVAL TIME IN VACCINATED AND NOT VACCINATED GROUPS

Source of variation	D. F.	Sx^2	Sxy	Sy^2	b	D. F.	$\frac{(Sxy)^2}{Sx^2}$	Sy^2aj (*)
Total	22	19,021.18	33,917.47	68,069.0		21		
Between groups	1	9.40	48.60	5,291.0		1		
Within groups (error)	21	19,011.78	33,868.87	62,778.0	1.781	20	60,336	2442
Control	10	9,082.62	19,216.32	40,843.0	2.116	9	40,653	190
Vaccinated	11	9,929.14	14,652.56	21,926.0	1.476	10	21,625	301

	Sy^2aj (*)	D. F.	Square Mean	F.	P.
Deviations from regression within groups.	2442	20			
Deviations from the two individual regressions.	491	19	25.8		
Differences between the regression in control and vaccinated groups.	1951	1	1951.0	75.62	***

(*) $Sy2aj = Sy2 - \frac{(Sxy2)}{Sx2}$

*** — P < 0,01



TIME OF DISEASE EVOLUTION (DAYS)

REGRESSIVE LINE ON THE PERCENTAGE OF DEAD ANIMALS AT THE TIME OF THE DISEASE EVOLUTION. COMPARISON BETWEEN CONTROLS AND BCG VACCINATED RATS

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