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T cell subsets in BCG induced lymphadenitis: an immunocytochemical investigation of blood and lymph nodes

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Özet

BCG'itisli olguların immunositolojik incelenmesi

BCG'itis tanısı konulan 36 çocuğun lenf düğümü büyüklüğüne, sertliğine ve fluktuasyon vermesine göre sınıflandırıldı. Lenfadenopatinin 2x2 cm. den küçük ve sert olduğu 8 olguda 3 ayda gerileme saptanırken, fluktuasyon veren 10 olgunun lenfadenopatisi cerrahi olarak drene edildi. Adenopatinin 2x2 cm. den büyük olduğu 18 olguda ise fluktuasyon versin veya vermesin cerrahi olarak tamamen çıkarıldı. İlaç önerilmedi. Olguların hepsi tamamen iyileşti.

7 olgunun periferik kan ve lenf düğümünde, T lenfosit alt grubu monoklonal antikorlarla (4-18 ay) incelendi. Lenf düğümünde histolojik olarak tipik kazeifikasyon gösteren epiteloid granuloma saptandı.

Lenf düğümünün kriyostat kesitlerinde immunoperoxidaz tekniği kullanılarak T lenfosit subpopulasyonunun dağılımı araştırıldı. Granüloma içinde ve etrafında saptanan T11+ hücrelerin büyük çoğunluğunu T4+ hücreler oluşturuyordu. T8+ hücreler granülomanın çevresinde seyrek olarak gözlenmekteydi. Lenf düğümünde T4+ /T8+ hücre oranının belirgin olarak arttığı gösterildi. periferik kandaki T4+/T8+ hücre oranı ise 1.34+0.53 (dağılımı 0.47-2.1) olarak belirlendi.

Anahtar kelime: BCG'itis

Summary

36 cases of BCG induced lymphadenitis were classified due to their enlargement, firmness or fluctuation. In 8 cases lymph nodes were smaller than 2x2 cm and firm which regressed in 3 months. Fluctuating lymph nodes drained surgically in 10 cases. In 18 cases whose lymph nodes bigger than 2x2 cm (either firm or fluctuating) exiced totaly. No medication was given. Healing was satisfactory.

T lymphocyte subsets in peripheral blood and axillary lymph nodes from 7 cases (range 4 to 18 months) were evaluated with monoclonal antibodies. Histologic examination of the excised lymph nodes revealed typical caseating epitheloid granulomas.

The distribution of T lymphocyte subpopulations in lymph node granulomas were studied in cryostat sections by an immunoperoxidase technique. T11+ cells were found both around and within the granulomas, T4+ lymphocytes represented the great majority of the T11+ cell population, both within and around the granulomas whereas T8+ cells were less numerous and usually confined to the periphery of the granulomas. Significant increases in the T4+/T8+ ratio was demonstrated in all lymph nodes. In the peripheral blood of all patients the ratio of helper to suppressor T cells was 1.34±0.53 (range 0.47-2.1).

Key words: BCG induced lymphadenitis

Introduction

BCG is a relatively safe vaccine and serious adverse effects are rare. Side effects commonly associated with BCG include ulceration at the vaccination site and lymphadenitis. These occur in 1 to 10 % of all vaccinees. Some strains have been associated with osteomyelitis (1 case per million doses administered) and lupoid reactions. Disseminated BCG disease, usually occur only in children with impaired cell-mediated immunity [12]. The incidence of lymphadenitis due to BCG vaccine is 5-8 percent in Turkey. It is well known that BCG induced lymphadenitis usually resolves spontaneously. Large regional lymphadenitis (2 cm.) has been a major problem for pediatricians. They lead to abscess formation with

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Table 1. Characteristic features of patients

Sex: 19 M. 17 F			Localisation of BCG induced lymphadenitis n: 36		
Age: 2-6 months	26	72.3 %	Axillary	30	83.3 %
7-24 months	10	27.7 %	Supraclavicular	6	16.6 %
			Both	3	8.3 %
PPD (5TU) n: 25			E.S.R n: 32		
			20 < 22		68.7 %
0-5 mm	0		> 20 10, 50± 20 mm/Hour		31.3 %
5-9 mm	8	32 %			
9-15 mm	9	36 %			
> 15 mm	8	32 %			
Isolation of Bacille n: 15 1 case (% 6.6)					
X-Ray: Normal	31	86 %			
Small interlober effusion	5	13.8 %			

Table 2. The outcome of BCG induced lymphadenitis.

The size of lymph Nodes	n	%	Outcome
Smaller than 2x2 cm			
Firm Node	8	22	Regressed
Fluctuating	10	27.7	Drainaged by surgeon
Bigger than 2x2 cm			
Firm Node	12	33.3	Excised totaly
Fluctuating	6	16.6	Excised totaly

chronic supuration for several months and the suggested therapies are controversial [2,9,11]. In the presented study, we report the outcome of 36 cases of BCG induced lymphadenitis and we characterize the T lymphocyte subsets using OKT MoAb in blood and also in tissue.

Materials and Methods

Patients: 36 patients with BCG induced adenitis (19 males, 17 females) were studied. The age distribution is shown in table 1. All patients have attended to our out-patient clinic with complaints of lymphadenitis. Supraclavicular lymphadenitis was abserved in 6 patients the remainder had axillary lymphadenitis. The results of tuberculin testing and eritrocyte sedimentation rates are shown in table 1. X-ray examination of the chest revealed small inter lobar effüsion in 5 cases which dissappeared after exicision or drainage of the lymph nodes. 36 cases of BCG induced lymphadenitis are classified due to their enlargement, firmness of fluctuation (Table 2).

Lymphocyte preparation: peripheral blood lymphocytes (PBL) were isolated from heparinized venous blood by the Ficoll-Hypaque sedimentation method. Blood were collected for study on the same day.

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Monoclonal antibody: OKT 11+ (T11+), OKT4+ (T4+), OKT8+ (T8+) were purchased from Ortho Pharmaceutical, New -Jersey, USA. OKT11+ Recognizes E rossetting cells including mature peripheral T lymphocytes, OKT4+ has been define the inducer/ helper T lymphocyte, whereas OKT8 defines the reciprocal supressor/cytotoxic population (6).

OKT11+, OKT4+ and OKT8+ monoclonal antibodies (50 ul) were added to cell suspension (50 ul) and left to incubate 4°C for 30 minutes. Cells were washed twice by centrifugation and resuspended in 50 ul of floresein conjugated goat antimouse IgG following 30 minutes at 4°C the cells were washed twice finally resuspended in one drop of 30 % glycerol in phosfate buffered saline, cells were examined on a flourescein microscobe. A minumum of 200 cells were examined for surface immunfluorescense.

Serial sections (6 to 7 mm thick) of frozen lymph nodes were cut on a cryostat, air dried at room temperature, at least for 12 hour, fix in cold acetone 10 minutes. Following acetone fixation the section were washed with PBS for 15 minutes followed by staining of different sections with T11+, T4+ and T8+ mouse antihuman monoclonal antibodies (DAKO PATS-Denmark). Following the initial incubation (30 minutes) with primary antibody the sections were washed multiple changes of PBS for 5 minutes and than incubated with link antibody for 30 minutes, sections were finally washed in PBS and incubated in APAAP (DAKO PATS, Denmark) for 30 minutes, colour reaction was performed using substrate solution and developed for 10 minutes. The sections were counterstained with Mayer's hematoxyclin for 15 minutes.

Table 3. T lymphocyte profile in patients with BCG induced lymphadenitis.

n:7 Leucocyte	Lymphocyte	T11	T4	T8	T4/T8
m 10.514	6769	39.1	33.7	27	1.34
Sd ± 2224	2113	14	8.5	9	0.53

Results

36 children were immunized with BCG vaccine in different centers. 28 infants were vaccinated during the newborn period and the rest of them at 2 to 15 months of age. Supraclavicular lymphadenitis was observed in 6 patients, the remainder had axillary lymphadenitis (Tablo 1). Tuberculin testing was performed to 25 cases (Table 1). Erintrocyte sedimentation rates were high (> 20 mm/hour) in 9 cases (Table 1). X-ray examination of the chest revealed small interlobar effusion in 5 cases which disappeared after surgical excision of the lymph nodes. Mycobacterium tuberculosis was isolated only in one case (among 15 cases) 36 cases of BCG induced lymphadenitis were classified due to their enlargement firmness or fluctuation. In 8 cases lymph nodes were smaller than 2x2 cm and firm which regressed in three months. Fluctuating lymph nodes were drained surgically in 16 cases. In 18 cases with lymph nodes bigger than 2x2 cm (either firm or fluctuating) total excision was performed. No medication was given other than cephalosporin to prevent secondary infection in 10 cases. Healing was satisfactory (Table 2).

Histologic examination of the excised lymph nodes revealed typical caseating epitheloid granulomas.

Peripheral blood T lymphocyte profile of cases (Range 4 to 18 months) are shown in table 3. In the peripheral blood of 7 patients the ratio of helper to supressor T cells was 1.34±0.53 (Range 0.47-2.1).

The distrubution of T lymphocyte subpopulation in lymph node granulomas were studied in cryostat sections by an immunoperoxidase technique. The distributional patern of the T cells showed that OKT 4+ cells were localized in around of granulomas whereas OKT 8+ cells were distributed predominantly in discontitinous rings around the granulomas. A similar distributional patern was observed in other hypersensitivity granulomas as tuberculosis and sarcoidosis [5,13].

Immunocytochemical typing of T cell subsets in frozen section of BCG lymphadenitis proved to be useful both in confirming the ratio between T cell subsets and in defining the distributional patern of OKT 4+ and OKT 8+ cells.

Discussion

BCG vaccine is an attenuated strain immunologically closely related to the virulent strain of Mycobacterium bovis. Marked differences in immunogenic and sensitizing potency of BCG strains were demonstrated over 20 years ago [3]. There is not a single scientifically defined entity known as BCG vaccine. There are rather different BCG vaccines, with varied immunizing potency in man [10].

Lymphadenitis due to BCG vaccine may occur approximately in 10 weeks but it may prolonge to 8-10 months. In our cases lymphadenitis were recognized by their parents within 2-3 month in 50 % of the cases. Lorber et al. suggested that isoniazid therapy is insufficient because it does not penetrate to the infected tissue [7]. Singh and Power have recommended eritromycin in the troublesome BCG lesions [9,11].

Çağlayan et al. suggested that the medical therapy may have some beneficial effect if used in those in whom lymph adenitis develops rapidly after vaccination. Total surgical excision is recommended to prevent spontaneous drainage and chronic suppuration in these rapidly evolving subjects [2].

We have chosen cephalosporings for the patients whose lesions did not improve within a week after surgical exsicion. Healing was satisfactory (Table 2).

Boyacıoğlu showed 11 % increased tuberculin reaction to BCG vaccine in her study population [1]. Youmans emphasized high susceptibility to tuberculosis in persons whose tuberculin resection is above 15 mm in diameter [15]. Our 8 patients tuberculin reactions were greater than 15 mm (Table 1), high erihtrocyte sedimentation rate was found in 4 patients and in one case small interlober effusion accompanied with the lymphadenitis.

Mycobacterium tuberculosis was isolated in only one case among 15. After excision the physical examination of the patient and the laboratory

tests for tuberculosis were within normal limits.

Our study demonstrated an involvement of cell-mediated immunity in BCG induced lymph adenitis. Evaluations of T cell subsets in peripheral blood revealed normal OKT 4+/OKT 8 ratios but on the other hand we found significant increases in the OKT 4+/OKT 8+ ratios in the sites of tissue with BCG adenitis.

Harada et al, in their study of pulmonary tuberculosis demonstrated that moduls the ratio of T4+/T8+ cells tended to be high compared to the normal. Several investigators showed moderate reduction in the ratio of 4+/T8+ in the blood of patients with sarcoidosis (13). Kiepiela et al found OKT 4+/OKT 8+ ratio ((1.04±0.06) in 20 healthy children aged between 5 months-8 years (median age 13 months) (6). The same author reported that patients with severe measles had a reduction in OKT 4+ and OKT 8+ cells number and observed decreased OKT 4+/OKT 8+ ratios.

In the peripheral blood of 7 patients the ratio of helper to supressor T cells was 1.34±0.53 (range 0.47-2.1). This findings is in agree with Kiepiela's T4+/T8+ ratio in healthy children.

Since we do not have normal healthy control values at the same age, we assesed the possible corelation between the variations of the T cell subsets as expressed by the T4+/T8+ ratio in the peripheral blood and the BCG lymph nodes of affected patients.

Conclusion

In patients with BCG induced lymph adenitis the OKT 4+/OKT 8+ ratio and distributional patern of T cells in the affected lymph nodes provided evidence of the hypersensitivity reaction like in the sarcoidosis and tuberculosis. A normal Helper/Supressor T cell ratio in peripheral blood of children is concomitant with the presence of normal cell-mediated immun response. Hypersensitivity reactions as hyperreactivity to PPD: small interlobar effusion and high eritrocyte sedimentation rate which improve after surgical procedure suggest that the adenitis due to BCG vaccine is result of excess antigenic stimulation and varied host-parasite relationship.

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