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Immunohistology of tuberculous adenitis in symptomatic HIV infection

JEN-YEE SHEN*, P. F. BARNES†, T. H. REA* & P. R. MEYER‡ *Sections of Dermatology and †General Medicine of the Department of Medicine and the ‡Department of Pathology of the University of Southern California, School of Medicine, Los Angeles, California, USA

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SUMMARY

Monoclonal antibodies and immunoperoxidase staining were used to characterize the cellular subpopulations in lymph nodes from 10 patients with tuberculous lymphadenitis, seven of whom had symptomatic human immunodeficiency virus (HIV) infection. $CD4^+$ cells were significantly fewer in nodes of patients with HIV infection than in those of immunocompetent patients. $CD8^+$ cells were distributed throughout the granuloma in patients with HIV infection, but confined to the periphery in normal hosts. Blastoid $Ta1^+$ cells, putatively antigen-reactive T lymphocytes, were seen in immunocompetent patients but not in those with HIV infection, suggesting that these cells fail to mature appropriately in the latter group. The immunopathological features noted above provide preliminary evidence that the cell-mediated immune response to tuberculosis is abnormal in patients with HIV infection, and may in part explain both the severe and the unusual manifestations of tuberculosis in these individuals.

Keywords HIV infection tuberculous adenitis T lymphocyte subsets

INTRODUCTION

Mycobacterum tuberculosis causes significant morbidity and mortality in patients with human immunodeficiency virus (HIV) infection, including those with the late complications of acquired immunodeficiency syndrome (AIDS) and the AIDS-related complex (ARC) (Pitchenik *et al.*, 1984; Louie, Rice & Holzman, 1986; Sunderam *et al.*, 1986). Among individuals with HIV infection, extrapulmonary tuberculosis, most commonly adenitis, is a predominant manifestation of infection. Miliary and progressive primary disease are also frequent manifestations of tuberculosis in this population, reflecting host inability to contain the mycobacterial pathogen. To improve our understanding of this important problem, we compared the immunopathological characteristics of tuberculous lymph nodes in immunocompetent patients and those with AIDS or ARC.

MATERIALS AND METHODS

Patient population.

We studied 10 individuals with culture-proven tuberculous adenitis, seen at the Los Angeles County-University of Southern California Medical Center between January 1984 and December 1985. Patients were classified as having AIDS or ARC according to standard CDC criteria (Centers for Disease Control, 1982, 1985). HIV-antibody testing was not routinely available at our hospital during the study period, and was not performed in our patients.

Correspondence: Thomas H. Rea MD, Section of Dermatology, USC School of Medicine, 2025 Zonal Avenue, Los Angeles, California 90033, USA.

Immunoperoxidase staining.

Lymph nodes were placed in OCT medium (Ames Co.), rapidly frozen in liquid nitrogen and stored at −70°C until sectioning. Cryostat sections 5 μm thick were cut, air dried, placed in reagent grade acetone at room temperature for 10 min just before immunostaining, allowed to dry and then hydrated in modified phosphate-buffered saline (PBS).

Murine monoclonal antibodies were used at concentrations pre-determined by checkerboard titrations. Each specimen was stained with the following antibodies: a $CD3^+$ pan-T-lymphocyte marker, Leu 4 (Becton Dickinson [BD]) at 1:400; a $CD4^+$ helper/inducer T-cell marker, Leu 3a (BD) at 1:150; a $CD8^+$ suppressor/cytotoxic T-cell marker, Leu 2a (BD) at 1:100; a monocyte/macrophage marker, Leu M3 (Coulter) at 1:150; anti-HLA-DR (BD) at 1:200; and a marker of specifically sensitized T lymphocytes, anti-Ta1 (Coulter) at 1:50. Controls consisted of omission of the primary antibody or use of irrelevant antibodies.

After acetone fixation and PBS hydration, slides were incubated sequentially with monoclonal antibody and peroxidase-conjugated anti-mouse IgG (Tago) at 1:20. Each incubation lasted 15 min, and 5-min PBS washes were performed between incubations. Aminoethyl carbazol and hydrogen peroxide were then added for 8 min. After 5 min of a tap-water

Table 1. Immunostaining in ten patients with tuberculous adenitis

Patient	Diagnosis	CD3$^+$(%) (Leu 4$^+$)	CD4$^+$(%) (Leu 3a$^+$)	CD8$^+$(%) (Leu 2a$^+$)	Ta1$^+$(%)	Leu M3$^+$	HLA-DR$^+$
1	AIDS	40	10	30	5	2$^+$	4$^+$
2	AIDS	20	5	15	5	3$^+$	4$^+$
3	AIDS	50	7·5	40	2·5	4$^+$	4$^+$
4	AIDS	45	25	25	7·5	2$^+$	4$^+$
5	AIDS	40	15	30	10	3$^+$	4$^+$
6	ARC	40	5	25	2·5	3$^+$	4$^+$
7	ARC	40	5	40	10	3$^+$	4$^+$
8	Normal	80	50	10	15	2$^+$	3$^+$
9	Normal	65	40	20	15	2$^+$	3$^+$
10	Normal	70	30	30	5	2$^+$	4$^+$

wash, slides were counterstained with Mayer's hematoxylin for 1 min, rinsed with water for 3 min, and mounted with glycerin-gelatin.

Positively stained cells were quantified as previously described (Modlin *et al.*, 1984). Briefly, the percentage of positive cells was estimated by comparing the number of stained cells to all cells in the granuloma. Estimates of two independent observers were averaged to obtain each percentage.

The Wilcoxon rank-sum test was used in statistical analysis.

RESULTS

Three patients had no clinical evidence of AIDS or ARC. Five patients developed opportunistic infections characteristic of AIDS, such as *Pneumocystis carinii* pneumonia, cytomegalovirus retinitis and esophageal candidiasis. These infections were diagnosed 4–11 months after tuberculous adenitis was noted in four patients, and concurrently with tuberculosis in one individual. Two patients had risk factors for HIV infection and had symptoms indicative of ARC. Lymph nodes in patients with AIDS and ARC were similar in immunopathological appearance. They were therefore combined for comparison with those from immunocompetent patients.

Upon staining with haematoxylin and eosin, all lymph nodes examined had characteristic granulomas with caseation necrosis. While more acid-fast bacilli (AFB) were seen in AIDS/ARC patients, there were no morphological differences between the granulomas from AIDS/ARC patients and those from immunocompetent individuals. The number and degree of differentiation of infiltrating macrophages (epithelioid cells) were similar in both groups. Though fewer lymphocytes were seen in the AIDS/ARC tissue, the pattern of their distribution was similar to that of the immunocompetent patients.

Immunostaining results in the 10 cases are shown in Table 1. Table 2 compares the findings in patients with AIDS/ARC with those in immunocompetent individuals. In lymph nodes from the three immunocompetent patients, CD3$^+$ lymphocytes (Leu 4$^+$ cells) formed the majority (72%) of the mononuclear cells involved in the granulomatous response. In the granulomas of AIDS/ARC patients, only 39% of the mononuclear cells were CD3$^+$ lymphocytes. Compared to immunocompetent patients, individuals with AIDS/ARC showed significantly fewer CD4$^+$ helper/inducer T lymphocytes (Leu 3a$^+$ cells), but similar numbers of CD8$^+$ suppressor/cytotoxic T cells (Leu 2a$^+$ cells).

CD4$^+$ cells were distributed throughout the granuloma in both groups (Fig. 1). CD8$^+$ cells were confined to the lymphocytic mantle aurrounding the epithelioid cells in the immunocompetent patients (Fig. 2), but were found throughout the granuloma in patients with AIDS/ARC. The percentages of Ta1$^+$ cells in AIDS/ARC and immunocompetent patients were similar (Table 2). However, half of the Ta1$^+$ cells had blastoid (large) nuclei in immunocompetent individuals, whereas in the

Table 2. Immunostaining in tuberculous adenitis with and without symptomatic HIV infection

	Symptomatic HIV infection ($N=7$)	Immunocompetent patients ($N=3$)	P value
CD3$^+$ (Leu 4$^+$)	39±3	72±4	0·02
CD4$^+$ (Leu 3a$^+$)	10±3	40±6	0·02
CD8$^+$ (Leu 2a$^+$)	29±3	20±6	0·27
Ta1$^+$	6±1	12±3	0·18

All percentages are expressed as the mean±s.e. of the mean.

Fig. 1. Granuloma from an immunocompetent patient with tuberculous lymphadenitis. Helper/inducer T lymphocytes are seen as small dark rings distributed throughout the granuloma.

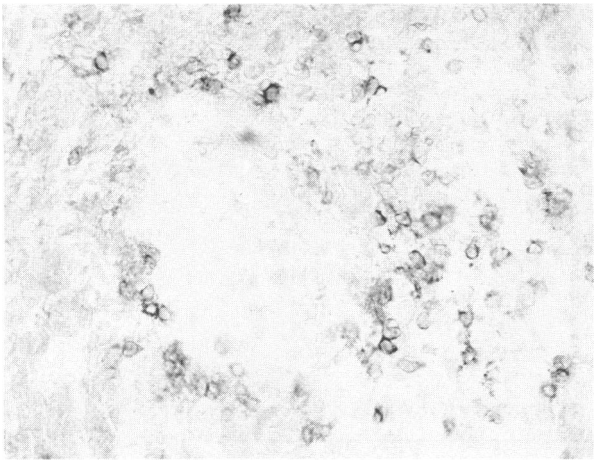

Fig. 2. Granuloma from an immunocompetent patient with tuberculous lymphadenitis. Suppressor/cytotoxic T cells, the small dark rings, are confined to the periphery of the granuloma.

AIDS/ARC patients, no cells with blastoid nuclei were seen. Staining with anti-Leu M3 and anti-HLA-DR yielded similar results in both groups.

DISCUSSION

Granuloma formation is the typical cell-mediated immune response to tuberculosis in normal hosts. In AIDS patients with *M. avium-intracellulare* infection, inflammatory infiltrate is minimal and granulomas absent or poorly formed, despite large numbers of AFB in tissue sections (Cohen *et al.*, 1983; Castella *et al.*, 1985). In the present series, the histological appearance of tuberculous lymph nodes in patients with AIDS or ARC was similar to that found in immunocompetent patients. Granulomas with caseation necrosis were seen in all cases. A potential explanation for this lies in the fact there is wide variation in the degree of defective cellular immunity among patients with AIDS or ARC. For example, individuals with *Pneumocystis carinii* have the most severely depressed CD4$^+$ lymphocyte counts, whereas those with Kaposi's sarcoma have less marked CD4$^+$ lymphopenia (Lane *et al.*, 1985). We and others have found tuberculosis to commonly precede the development of AIDS (Pitchenik *et al.*, 1984; Castella *et al.*, 1985; Sunderam *et al.*, 1986; Barnes & Arevalo, 1987). This is presumably because *M. tuberculosis* is a more virulent human pathogen than organisms such as *M. avium-intracellulare* and *P. carinii*, affecting patients with HIV infection at an earlier stage of immunodeficiency (Pitchenik *et al.*, 1984; Castella *et al.*, 1985; Blaser & Cohn, 1986; Barnes & Arevalo, 1987). Preservation of granuloma formation probably reflects this less severe defect in cellular immune function.

Immunostaining demonstrated several differences between adenitis in patients with AIDS or ARC and immunocompetent hosts. The decrease in CD4$^+$ lymphocytes, but normal number of CD8$^+$ cells is consistent with the selective infection and destruction of CD4$^+$ lymphocytes by HIV (Dalgleish *et al.*, 1984; Klatzmann *et al.*, 1984).

Some of the differences between lymphocyte distribution in the nodes of AIDS/ARC patients and immunocompetent individuals parallel those noted in tuberculoid and lepromatous leprosy. In tuberculoid leprosy, host cell-mediated immunity is vigorous, whereas in lepromatous leprosy, the cellular response is very poor. We have found CD8$^+$ cells to be confined to the periphery of the granuloma in tuberculoid leprosy, whereas they are scattered throughout the tissue in lepromatous leprosy (Modlin *et al.*, 1983). Parallel changes were noted in tuberculous adenitis in the present study, CD8$^+$ cells being concentrated in the mantle in immunocompetent patients, but distributed throughout the granuloma in symptomatic HIV infection. We have been unable to demonstrate a suppressor effect of CD8$^+$ cells derived from lesions of tuberculoid leprosy, and postulate that they may represent cytotoxic T lymphocytes that limit the spread of *M. leprae* (Modlin *et al.*, 1986). One might speculate that the CD8$^+$ cells in tuberculous adenitis play a similar role, surrounding the granuloma and preventing spread of mycobacterial infection. In our AIDS/ARC patients, the presence of CD8$^+$ cells throughout the granuloma, rather than in the periphery, may reflect abnormal organization and/or function of cytotoxic T cells in patients with symptomatic HIV infection. The nature and significance of this abnormality remain unclear at this time.

Ta1$^+$ cells are thought to represent a subset of antigen-reactive T lymphocytes that include memory cells (Fox *et al.*, 1984; Hafler *et al.*, 1986). In tuberculoid leprosy, the nuclei of Ta1$^+$ cells are often blastoid in appearance, whereas Ta1$^+$ cells in lepromatous leprosy invariably have small nuclei (Shen *et al.*, 1987) suggesting that antigen-reactive T lymphocytes fail to mature in the latter disease. The absence of blastoid nuclei among Ta1$^+$ cells in tuberculous lymph nodes from individuals with AIDS/ARC suggests a parallel dysfunction in patients with symptomatic HIV infection.

In summary, in tuberculous adenitis, we found subtle but potentially important immunohistological differences between lymphocyte subpopulations in patients with and without symptomatic HIV infection. Though granuloma formation may indicate partially effective cellular immunity, the relative depletion of CD4$^+$ cells, lack of restriction of CD8$^+$ cells to the mantle region, and absence of blastoid Ta1$^+$ cells, provide preliminary evidence that the cell-mediated immune response to *M. tuberculosis* is abnormal. Defective cellular immunity may in part explain the predilection of this population to severe and unusual manifestations of tuberculosis.

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