

Suppression associated lymphocyte markers in lesions of sarcoidosis

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ABSTRACT The presence within the epithelioid granulomas of sarcoidosis of lymphocytes of the "helper" (T4 positive) phenotype suggests that, as in granulomatous diseases of known aetiology, active cell mediated immunity plays a part in the disease. It has, however, been noted that T8 positive lymphocytes are also found on occasion in the granulomas. The presence of cells bearing markers associated with suppressor activity in various lesions of sarcoidosis has been investigated with a range of monoclonal antibodies. T4 positive cells were present in all granulomas; T8 positive lymphocytes were present within the epithelioid cell areas in proportions that varied from 5 to 55/100 of T4 cells. Other lymphocyte markers associated with suppression (Leu8 and SN130) were very rare on lymphocytes within granulomas but common on both T4 positive and T8 positive lymphocytes in the perigranulomatous mantle, which suggests that expression of these markers was down regulated in the granuloma or that cells bearing them are unable to migrate into the granuloma. Nevertheless, suppressor cell mechanisms could mediate the spontaneous resolution of most cases of sarcoidosis, and the presence or absence of cells bearing suppression associated phenotypes in the granuloma or its mantle may have prognostic importance.

Introduction

The division of lymphocytes into B cells (which produce immunoglobulins) and T cells (concerned with cellular immune responses) is well recognised, but it has become clear that the latter group can be further subdivided in terms of both antigenic phenotype and in vitro function. T cell functions include "helping" B cells to produce immunoglobulins and inducing cell mediated responses, and the cells that do this ("helper-inducer" T cells) bear a membrane antigen recognised by the monoclonal antibody OKT4 or equivalents (T4 positive). Cytotoxic and suppressor functions in vitro on the other hand are associated with an antigen recognised by the antibody OKT8 (T8 positive). Further functional and phenotypic subdivisions of both T4 positive and T8 positive cells can, however, be made. Paradoxically, some "helper-inducer" cells actually induce suppression of immune responses. This activity lies within the T4 positive subset, and is

found in a population also labelled by newer monoclonal antibodies such as 2H4 or anti-Leu8.^{1,2} The remaining T4 positive cells, which are 2H4 negative or Leu8 negative and do not induce suppression, have been called "true helper" cells. The antibodies 2H4 and anti-Leu8, which label distinct subpopulations¹ of lymphocytes, identify subsets of T8 positive cells and B cells as well as T4 positive cells.

It is tempting to equate phenotype with function so that pathological mechanisms in diseased tissues might be elucidated. The granulomas that characterise sarcoidosis contain lymphocytes, scattered among the macrophage derived epithelioid cells, although the compact granulomas are often also accompanied by an adjacent or encircling "mantle" of lymphoid and other mononuclear cells. It has been observed that T lymphocytes among the epithelioid cells are of the T4 positive subset^{3,4} and are Leu8 negative.⁵ The T suppressor-cytotoxic phenotype (T8 positive) is said to be restricted to marginal or mantle regions.^{3,4} Figure 1 shows an example of this distribution. Although the "true helper" phenotype of the granuloma lymphocytes does not guarantee that they are actively promoting an immune response, such a role would at least be consistent with experimental models of gran-

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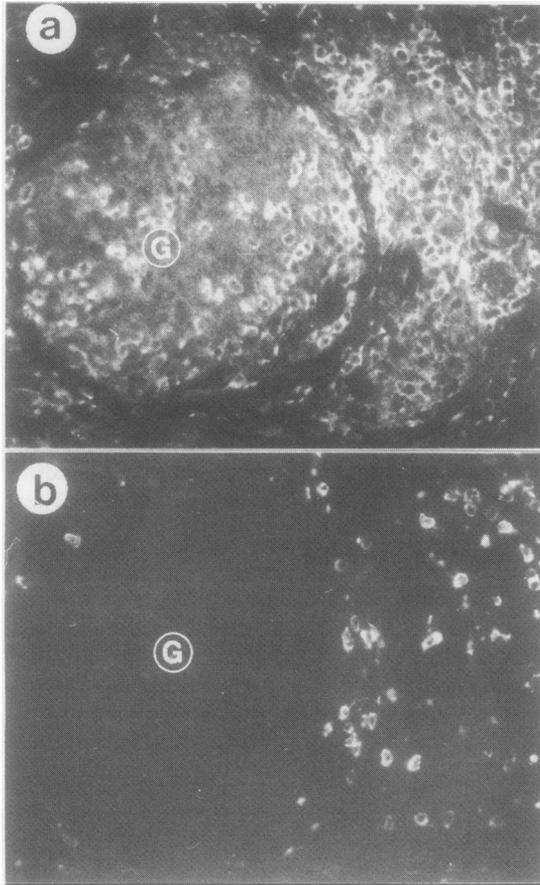


Fig 1 Cryostat section showing a positive Kveim response: (a) T4 positive cells identified with OKT4b and TRITC-anti IgM; (b) T8 positive cells identified with RFT8 and FITC-anti IgG in the same field. Note that only T4 positive cells are found in the granuloma (G), but both T4 positive and T8 positive cells are present around it.

ulomatous disease, in which hypersensitivity mediated by T cells is necessary for the formation of epithelioid granulomas.⁶

We have observed, however, that T8 cells are sometimes also present within granulomas. Animal models show the existence of spontaneous reduction in granulomatous inflammation mediated by suppressor type T cells.⁷ The spontaneous resolution of many cases of sarcoidosis could be due to a similar process and the presence or absence of suppressor cells could have prognostic significance. We have therefore examined a range of lesions of sarcoidosis with monoclonal antibodies, to determine the prevalence and phenotype of cells bearing markers associated in

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vitro with suppressor activity, notably those recognised by anti-T8, anti-Leu8, and a 2H4 equivalent, SN130.

Methods

Biopsy specimens were obtained from 14 patients with sarcoidosis (two lymph nodes, six skin lesions including four positive Kveim responses, six transbronchial biopsy specimens). All contained well defined epithelioid granulomas; perigranulomatous mononuclear infiltrates varied in extent. Cryostat sections (6 μ m) were studied with a range of monoclonal antibodies. These reagents and their functional associations are shown in the table. UCHT1 was a gift of Dr P Beverley; OKT4B was obtained from Ortho Diagnostics (Raritan, New Jersey, USA), and anti-Leu8 from Becton-Dickinson (Mountain View, California, USA). A new monoclonal antibody, SN130, was produced from a mouse immunised with suspensions of the two lymph nodes used in the study. It identifies subsets of B and T lymphocytes and subdivides both T4 positive and T8 positive cells; in tissue, labelled cells have a distribution identical to those identified by 2H4. SN130 blocks binding of phycoerythrin conjugated 2H4 (Coulter Immunology, Hialeah, Florida, USA) to tissue and lymphocyte suspensions. Combinations of T cell markers were identified with IgG monoclonals and OKT4B or RFT8 (IgM) as first layer, and class specific fluorescein (FITC) and rhodamine (TRITC) conjugated goat anti-mouse immunoglobulin (Southern Biotechnology Associates, Birmingham, Alabama, USA) as second layer. Both positive and negative controls were used routinely. Marker frequencies are derived from comparison with a minimum of 100 T4 positive cells, but up to 300 were counted when the size of the biopsy specimens permitted this. The coefficients of variation when the same specimen was stained and counted 10 times were 8% for UCHT1, 12% for RFT8 and anti-Leu8, and 17% for SN130.

Results

T cells were present in all granulomas. Perigranulomatous areas contained T cells and occasional B cells; as in normal lymph nodes, B cell follicles were present in nodes containing granulomas, and they were also seen in one skin lesion. The frequencies of various T cell markers in granulomas and perigranulomatous areas, expressed as percentages of T4 positive cells in the same zones, are shown in the table.

All the granulomas contained numerous T4 positive cells. In most lesions T8 positive cells were relatively rare within granulomas (fig 1). In all but two cases there were fewer T8 positive cells per 100 T4 positive

Reactions of lymphocytes within and around granulomas to various monoclonal antibodies

Antibody (similar reagents)	Specificity and functional associations	Mean (range) frequency of positive cells*	
		Within granulomas	Perigranulomatous areas
B cell markers RFB4, RFB6 ⁸	B cells	None	Not assessed
T cell markers UCHL1 ⁸ (= OKT3)	Most mature T cells	129 (109–153)	133 (106–170)
OKT4B ⁹	Subset of T cells associated with helper/inducer function	100	100
RFT8 ⁸ (= OKT8)	Subset of T cells associated with cytotoxic-suppressor function	25 (5–55)	53 (17–111)
Anti-Leu8 ²	Subsets of B, T4+, and T8+ cells; T4+ -Leu8+ cells include inducers of suppression; T4+ -Leu8-cells are "true helpers"	0.8 (0–2)	18 (5–40)
SN130 (= 2H4 ¹)	Subsets of B, T4+, and T+ cells; distinct from Leu8; T4+ -2H4+ cells also include inducers of suppression	1.1 (0–3)	21 (5–40)

*All values expressed as positive cells/100 T4+ cells; in lymph node lesions perigranulomatous lymphoid tissue may not be pathological.

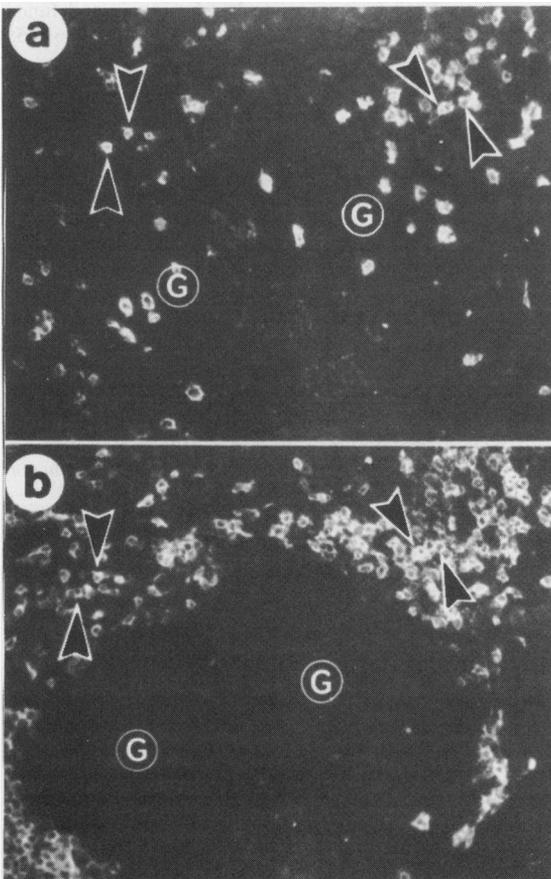


Fig 2 Cryostat section of a lymph node affected by sarcoidosis: (a) RFT8 positive cells identified with RFT8 and TRITC-anti IgM; (b) SN130 positive cells identified with SN130 and FITC-anti IgG in the same field. Note that several T8 positive cells are found within these granulomas (G), but cells labelled by both antibodies (examples arrowed) are found only around and not within granulomas.

cells within than around the granuloma. The mean frequency of each cell within granulomas (table) corresponds to a ratio of T4 positive to T8 positive cells of 4.0. In four of the 14 cases, however, the T4:T8 ratio was 3.0 (the upper limit for normal peripheral blood T cells) or less. When they were numerous within a granuloma T8 positive cells were distributed throughout the granuloma and not only at the edge of the epithelioid cell area. In the perigranulomatous infiltrates T4 positive and T8 positive cells were always present. In only three cases did a low frequency of T8 cells cause the T4:T8 ratio to be 3.0 or more (two transbronchial biopsy specimens with perigranulomatous T4:T8 ratios of 5.8 and 6.1, and one Kveim lesion, 3.3).

Within the granulomas neither T8 positive nor T4 positive populations were labelled by anti-Leu8 or SN130. Both these markers, however, were present in perigranulomatous infiltrates on T4 positive, T8 positive, and B cells. Figure 2 shows T8 positive cells in a lymph node lesion and the relative distribution of SN130 positive cells.

Discussion

We have confirmed the invariable presence and usual predominance within sarcoid granulomas of lymphoid cells whose phenotype (T4 positive, Leu8 negative, SN130/2H4 negative) corresponds to that of "true helper" T cells in vitro. With respect to cells bearing suppression associated markers, three observations deserve comment. Firstly, while T8 positive (suppressor-cytotoxic) cells do not appear to be essential, as granulomas in many lesions contained very few such cells, in other cases the T4 positive cell predominance was less. This was not simply a function of the age of the lesion, as positive Kveim test granulomas showed similar variation to that of spontaneous granulomas. Nevertheless, only single lesions from each subject were examined, and possibly there was variation between different lesions within an individual.

Secondly, however, neither T4 positive nor, when present, T8 positive cells in granulomas bore the Leu8 or SN130/2H4 markers. As with T4 positive cells, these markers are found on subpopulations of T8 positive cells, and in the case of Leu8 suppression in vitro requires both Leu8 positive and Leu8 negative subpopulations.¹⁰ If this requirement exists in vivo, the T8 positive populations found in some granulomas may be functionally insufficient to suppress immune responses. Thirdly, perigranulomatous T cell infiltrates contained T4 and T8 cells bearing markers (Leu8 and SN130) which were excluded from the granuloma or whose expression was down regulated within it. The two transbronchial biopsy specimens in which perigranulomatous T4:T8 ratios were particularly high may be areas of active granuloma formation, as suggested by Semenzato⁵; but in most cases the differences between granuloma and perigranulomatous mantle suggest that the pathological processes within them are distinct.

If cellular phenotypes are indeed relevant to their functional activity, the presence of these suppression associated lymphocyte markers, whether in granuloma or mantle, suggests that immune responses in sarcoidosis may be subject to secondary regulation. Such variation in immunoregulation is potentially important, as it could determine whether established granulomatous inflammation resolves spontaneously or progresses to chronic or fibrotic disease.

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