STUDIES ON THE IMMUNOPATHOLOGY OF TUBERCULOSIS

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In this presentation, two aspects concerning the immunopathology of tuberculosis at the microanatomical level will be considered. The material for this presentation is drawn from our studies on tuberculous lymphadenitis (450 cases) and skin tuberculosis (270 cases).

The first question to be considered is 'when is it necessary to demonstrate the components of *M.tuberculosis* in the tissues?' The second is the question of the involvement of humoral immune factors associated with some of the histological manifestations of tuberculosis.

Demonstration of the components Of *M. tuberculosis*

There are three clinical situations where it is necessary to visualise the components (either as antigens or as nucleic acids) of *M.tuberculosis* in the affected tissues.

1. Establishment of Diagnosis

Histopathologically, the signature of tuberculosis is usually unmistakable. The usual picture of a well-formed, epithelioid cell, giant cell, lymphocyte, plasma cell granuloma with a central caseation necrosis is often enough to confirm the clinical diagnosis of tuberculosis in an endemic country.

However, in about 25% of the cases, variant forms may be seen. These may include a well-formed, non necrotic, epithelioid cell granuloma at one end and a poorly-organised granuloma with vacuolated macrophage and necrosis without caseation at the other end. In such cases, demonstration of bacilli either by Ziehl-Neelson (Z-N) staining or culture for *M tuberculosis* would be helpful in establishing the diagnosis. Nevertheless, in about 40% of these cases, bacilli are usually not seen using Z-N staining and 25% of these are culture negative for *M. tuberculosis.* It is likely that demonstration of the bacillary components in such cases using probes which are specific for the organism will help the pathologist to arrive at the diagnosis of tuberculosis in such atypical presentations.

2. Establishment of the aetiology of granulomatous inflammation:

Identification of the nucleic acids of *M. tuberculosis* in granulomatous lesions has helped to identify the aetiological agent in two conditions.

a. Tuberculids: Erythema induratum and papulonecrotic tuberculids occurring in the skin are rare conditions traditionally attributed to a tuberculous aetiology. A casealing granuloma is often seen histologically and this has been considered to be a manifestation of tubcrculous hypersensitivity with the primary lodgement of the bacilli elsewhere in the body as *M. tuberculosis* cannot be cultured from these lesions.

Recently, the tuberculous aeliology of these lesions has been confirmed by Degitz and his colleagues (1993). Using a 383bp fragment of the mycobacterial gro EL gene amplified by primers TB1 and TB2, the tuberculous actiology of this granulomatous lesion has been identified by these workers.

b.Sarcoidosis: This forms an important differential diagnosis both clinically and histologically while a diagnosis of tuberculosis is actively considered. The aetiology of this condition has long remained a matter of speculation. However, several investigators have demonstrated *M. tuberculosis* nucleic acids in these lesions (reviewed in Mangiapan and Hance, 1995). It is possible that the granulomatous inflammation in sarcoidosis is an allergic manifestation caused by fragmented *M. tuberculosis.*

3. Establishment of resolution

Demonstration of the presence of *M. tuberculosis* antigen(s) may be of use in one more clinical situation. It is sometimes seen that in patients with tuberculous lymphadenitis. the lymph node does not decrease in size completely after a full course of anti tuberculous therapy (ATT). In such situations, biopsy of the node has shown that while a residual granuloma is still present, *M. tuberculosis* is neither seen on ZN staining nor can it be cultured. Demonstration of *M. tuberculosis* antigen will be of use in deciding whether the granuloma is still active and therefore require a further course of ATT.

Experiments were therefore conducted in the guinea pig to observe the resolution of granuloma *vis a vis* intact bacilli and *M. tuberculosis* antigen. Killed *M. tuberculosis* (2×10^7) was injected intradermally and groups of four animals were sacrificed at various time points from 611 to 12 weeks and the injected site was removed. In another experiment, live *M. tubercu1osis* (2×10^7) was injected intramuscularly and the animals were sacrificed from 4 to 44 weeks and their spleen, liver and lung were obtained. These, along with the skin specimens were evaluated for the presence of granuloma, acid fast bacilli (AFB) and *M. tuberculosis* antigen(s). Tuberculous antigen was demonstrated using polyclonal anti *M. tuberculosis* antiserum in an indirect immunoperoxidase method.

In all the four organs sites examined, stainable bacilli were the first to disappear followed by *M. tuberculosis* antigen and finally only the granuloma resolved. Further, it was found that the splenic culture of the infected guinea pig for *M. tuberculosis* became

negative long before the disappearence of the antigen. It is therefore concluded that the presence of granuloma in the absence of antigen in a clinical specimen from a residual lesion probably indicates that the granuloma is in the resolving stage and that the patient may not require further ATT.

Whither R cell responses in tuberculous granuloma?

A detailed analysis of the cellular profile of the histology of tuberculous lymphadenitis (TBL) and cutaneous tuberculosis (CTB) revealed the following:

1. A spectrum of responses varying from a well-organised, non necrotic epithelioid cell granuloma (Hyperplastic) through a caseating, epithelioid cell granuloma (Reactive) to a poorly-organised, macrophage granuloma with noncaseating necrosis (Hyporeactive and Nonreactive) was observed.

2. In cutaneous tuberculosis, the histology correlates with the clinical spectrum of venucosa cutis, lupus vulgaris and scrofuloderma. Verrucosa cutis is characterised by a non necrotic, epithelioid cell granuloma while in scrofuloderma, necrosis is a hall mark of its histology. In lupus vulgaris, minimal necrosis was seen in 9 out of 127 cases.

3. Plasma cells and B cells were found in moderate numbers in all these granulomata; these were seen more often when there was necrosis (Table 1).

4. There were no differences in the response to purified protein derivative (RT23) at 72 hours amongst the various histological groups within TBL or within CTB (Table 2).

TABLE 1: DISTRIBUTION OF PLASMA CELLS AND B LXMPHOCYTES IN TUBERCULOUS LYMPHADENITIS AND SKIN TUBERCULOSIS (Mean±95% confidence interval)

	TYPE	PLASMA CELLS	B LXMPHOCYTES
LYMPH NODE			
	HYPERPLASTIC	0.7±0.3	6.8±1.2
	REACTIVE	7.5±0.6	12.1±0.9
	HXPO REACTIVE	12.4±2.1	17.3±1.1
	NON REACTIVE	18.5±5.8	19.4±1.5
SKIN			
	LUPUS VULGARIS	4.4±0.9	10.1±1.3
	VERRUCOSA CUTIS	4.0±1.2	13.1±8.1
	SCROFULODERMA	16.2±1.6	18.4±5.6

TABLE 2: MANTOUX REACTION AT 48h TO PPD

TYPE	<10mm	>10mm	MEAN	95% C.I.
LYMPH NODE				
HYPERPLASTIC	9	55	25.5	20.8-28.3
REACTIVE	24	135	26.8	24.1-29.5
HYPOREACTIVE	8	41	25.4	22.8-27.9
NONREACTIVE	4	7	27.3	15.2-34.3
SKIN				
LUPUS VULGARIS	11	72	16.9	14.9-18.9
VERRUCOSA CUTIS	11	61	17.9	15.7-20.1
SCROFULODERMA	2	17	19.2	15.4-23-0

It is possible that these represent a polarisation of the T lymphocyte responses into Type 1 and Type 2 patterns as has been demonstrated in leprosy lesions (Yamamura *et al,* 1991; Salgame *et al,* 1991). The patterns of T cell cytokine response from human peripheral blood mononuclear cells to PPD have been studied (Del Prete *et al,* 1991; Barnes *et al,* 1992). A shift from Thl to Th2 response occurs when the acute stage of' the disease leads onto the chronic stage in the lung tissue of a murine model for tuberculosis (Hemandez-Pondo *et al,* 1996).

The cytokine profile of the lymphocytes in human tuberculous granuloma is yet to be characterised fully. It is tempting to argue that the histological spectrum observed in TBL and CTB may represent polariscd T cell responses. However, the Mantoux reactions in these patients do not show differences amongst the various histological groups suggesting that a clear cut polarisation as seen in leprosy lesions may not be demonstrable in tuberculosis though this needs to be looked at in greater detail.

The presence of moderate numbers of B cells and plasma cells in lesions where there is necrosis needs to be explored further. A number of possibilities exist to explain their presence and some of them are discussed here.

The plasma cells arising from B cells can produce antibodies and along with the fragmented *M. tuberculosis* antigen can form in situ immune complexes. These then can not only initiate but also determine the type of granuloma formed (Spector and Heesom, 1969; Ridley *et al.* 1982). Futher, Campa *et al* (1989). have shown that anti idiotypic B cells against *M. tuberculosis* can modulate both granuloma formation and delayed hypersensitivity reaction to PPD. Antigen-specific B lymphocytes are potent antigen presenting cells. It is known that B cells presenting antigen to naive T lymphocyte can result in tolerance whereas if they present antigen to sensitized T cells, they can be immunized (reviewed in Matzinger, 1994).

Recommendations

In view of the foregoing. three major areas of research concerning the immunopathology of tuberculosis are recommended.

1. Development of reagents to identify *M. tuberculosis* antigen(s)/nucleic acid(s) in tissues suspected to be of tuberculous pathology though with a caveat. It is advisable to use these reagents only when there is a *prima facie* case for doing so. In other words, the lesion should have a granuloma and bacilli not demonstrable either using Z-N staining or by culture for the organism.
2. Delineation of the in situ cytokine responses in different histological forms of tuberculosis.
3. Elucidation of the role of humoral immune responses, especially that of the B lymphocytes in the histological manifestations of the disease.

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