Review Article

Immune Responses to Mycobacterial Antigens in Sarcoidosis: A Systematic Review

D. Gupta[1], R. Agarwal[1], A.N. Aggarwal[1], and Indu Verma[2]

Departments of Pulmonary Medicine[1] and Biochemistry[2], Postgraduate Institute of Medical Education and Research, Chandigarh, India

ABSTRACT

From the time sarcoidosis has been described, there has always been a viewpoint that the disease is in some way related to tuberculosis (TB). Sarcoidosis is a granulomatous disease, which is likely a result of continued presentation of a poorly degradable antigen. *Mycobacterium tuberculosis* has been a very strong contender for this antigen. Besides the molecular studies demonstrating mycobacterial deoxyribonucleic acid (DNA) in the sarcoid tissue, assessment of immune responses against mycobacterial antigens provides a useful tool to study the role of mycobacteria in the pathogenesis of sarcoidosis. We reviewed the studies focussing on T-cell and B-cell responses to tubercular antigens in patients with sarcoidosis. Pooled data from various studies does provide a suggestive, though not unequivocal evidence in favour of mycobacteria as a cause of sarcoidosis. These findings not only reinforce the possible pathogenic role of mycobacterial antigens in sarcoidosis, but at the same time also limit the clinical utility of molecular and serological studies based on mycobacterial antigens in the differential diagnosis of TB from sarcoidosis, particularly in a country with high endemicity for TB.
[Indian J Chest Dis Allied Sci 2011;53:41-49]

Key words: Sarcoidosis, *Mycobacterium tuberculosis*, IGRA, T-cell responses, B-cell responses.

INTRODUCTION

Sarcoidosis is a granulomatous disease, which like all other granulomatous diseases is most likely a result of continued presentation of a poorly degradable antigen.[1] In a quest to identify this 'poorly degradable antigen', numerous aetiologic agents have been incriminated, both infective and non-infective.[2] Non-infective agents have been implicated because of their epidemiologic association,[3] but have not stood the test of time.[4] Examination of the sarcoid granuloma with an electron microscope and immunohistochemical techniques has identified structures similar to organisms, such as *Leptospira* species, *Mycoplasma* species, and *Propionibacterium* species.[2] Other micro-biological agents that have been implicated from time to time include herpes virus, retrovirus, *Chlamydia pneumoniae*, *Borrelia burgdorferi*, *Rickettsia helvetica*, and finally *Pneumocystis jirovecii*.[2,5] However, one of the strongest contender remains the *Mycobacterium*.[6-8]

The probability of a causative link between TB and sarcoidosis has intrigued physicions.[9] However, the inability to identify mycobacteria by histologic staining or culture from pathologic tissues of sarcoidosis continues to be one of the strongest arguments against a potential role for mycobacteria. A recent meta-analysis[10] showed that mycobacterial deoxyribonucleic acid (DNA) was present in 30% of sarcoid samples, although individual studies had reported detection rates from zero percent to 50 percent. It needs to be emphasised that most of these studies were published from countries with a low prevalence for TB. If indeed mycobacteria are aetiologically linked to sarcoidosis, then the detection rates for mycobacterial DNA in sarcoid samples should be higher in countries with a high prevalence of TB. In a recent prospective, case-control study from India[11] aimed at detection of mycobacterial DNA in patients with sarcoidosis, we have demonstrated mycobacterial DNA by polymerase chain reaction (PCR) for 65 kDa protein gene in 48% of samples (bronchoalveolar lavage [BAL] or biopsy) from freshly diagnosed patients of sarcoidosis. This reinforces the hypothesis of mycobacteria as a causative agent for sarcoidosis.

The factors that favour mycobacteria as a trigger for sarcoidosis include histopathological appearances of the granulomas,[1] reports of mycobacterial disease either existing before, during or after sarcoidosis,[12,13] and the finding of mycobacteria in occasional granulomas of sarcoidosis.[14-16] Passage experiments have also suggested that mycobacteria with

[Received: October 21, 2010; accepted: November 16, 2010]

Correspondence and reprint requests: Dr Dheeraj Gupta, Additional Professor, Department of Pulmonary Medicine, Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh-160 012 (India); Telephone: 91-172-2756823, Telefax: 91-172-2748215; Email: dheeraj1910@gmail.com

characteristics of *Mycobacterium tuberculosis* may be the incriminating agent.[17-20] It has also been suggested that the organism might exist in a cell wall deficient L-form and may be difficult to isolate.[21] Recent studies on humoral immunity to mycobacterial antigens from sarcoidosis patients have renewed interest in a potential role of mycobacteria in sarcoidosis.[22] Assessment of immune responses against microbial proteins is a useful tool to study the role of mycobacteria in the pathogenesis of sarcoidosis.

In this review, we look into the immunopathogenesis of sarcoidosis in brief and review the studies focussing on T-cell and B-cell responses to tubercular antigens in patients with sarcoidosis. For the purpose of this review, we have performed a systematic search of the electronic databases — MEDLINE and EMBASE — for relevant studies published from 1965 till current date using the free text term: sarcoidosis. In addition, we reviewed our personal files. A total of 100 articles were reviewed in detail for the purpose of this review.

IMMUNOPATHOGENSIS OF SARCOIDOSIS

A non-caseating granuloma is the hallmark of sarcoidosis. The granulomatous reaction is a protective response to the inciting agent. It limits inflammation and protects the tissue. Granuloma formation briefly involves four stages. In the first stage, the inciting antigen comes in contact with antigen processing cells (APCs), which are usually the alveolar macrophages. These APCs then interact with CD4+ T-lymphocytes in the second stage to initiate the formation of a simple granuloma. In the third stage, there is a further recruitment of CD4+ T-lymphocytes and type 1 and type 2 helper responses are elicited, that lead to the final or fourth stage of formation and maintenance of complex granulomas. Macrophages differentiate to form epithelioid cells under the influence of cytokines. These epithelioid cells fuse to form multi-nucleated giant cells and gain secretory properties. Granulomas secrete many humoral substances including calcitriol and angiotensin converting enzyme. These events, however, are dependent on a susceptible genetic background described by a variety of functional polymorphisms.[23,24]

Extensive research over the past two decades has helped to delineate the sequence of events in the immunopathogenesis of sarcoidosis. The earliest event is the accumulation of CD4+ helper T cells and release of interleukin (IL)-2 in the alveoli and interstitium.[25-27] This is followed by a progressive and selective oligoclonal expansion of αβ T cells.[28] There is an increased *in situ* production of Th1 cell-derived cytokines (IL-2 and interferon [IFN]-γ) during granuloma formation.[29,30] The alveolar macrophages in sarcoidosis have immense secretory properties and there is an increased release of macrophage-derived cytokines (IL-1, IL-6, IL-8, IL-15, tumour necrosis factor [TNF]-α, IFN-γ, granulocyte macrophage-cerebrospinal fluid [GM-CSF]) and chemokines (RANTES, MIP-1α, IL-16). Most of these cytokines favour granuloma formation and lung damage.[31-34] The progression and maintenance of the granulomatous reaction is also favoured by the accumulation of these monocyte-macrophages with antigen-presenting cell capacity and expressing increased levels of activation markers (human leukocyte antigen [HLA]-DR, HLA-DQ, CD71) and adhesion molecules (CD49a, CD54, CD102). The chemo attractant cytokines, such as IL-8, IL-15, IL-16 and RANTES, recruit the CD4+ T cells from the peripheral blood to the site of inflammation, while IL-2 induces an *in situ* proliferation of these cells.[35,36] The mechanism(s) that result in spontaneous resolution or progression to chronic disease and fibrosis are unclear but may be linked to host susceptibility and genetic factors. The sarcoid granulomatous inflammation is characterised by an altered balance of Th1/Th2 responses with a dominant expression of Th1 cytokines (IFN-γ and IL-2) with low levels of expression of Th2 cytokines including IL-4 and IL-5.[27,37] The IL-12 contributes to proliferation of activated T cells in early disease. Elevated levels of IL-6 and IL-8 are reported in the BAL fluid of active sarcoidosis, and these may modify the disease process. The IL-15 may aid in the proliferation of T- and B- cells.[38] The IL-12 and IL-18 are also increased in the lungs of sarcoidosis, and they stimulate IFN-γ production.[27,39] Transforming growth factor (TGF)-β is an inhibitor of IL-12 and IFN-γ production. Its production is increased in patients undergoing remission of sarcoidosis, suggesting a key role for TGF-β in the down regulation of the granulomatous inflammation of sarcoidosis.[40]

IMMUNE RESPONSES AGAINST MYCOBACTERIAL ANTIGENS

Along with the detection of mycobacterial DNA in the sarcoid granulomas as described above, mycobacterial peptide fragments have also been demonstrated within sarcoidosis granulomas and both T-cell-mediated immune responses and B-cell-mediated humoral immune responses against these antigens have been reported. Identification of genes in the *M. tuberculosis* genome has enabled the detection of proteins such as 6-kDa early secreted antigenic target (ESAT-6) and the 10-kDa culture filtrate protein (CFP-10) encoded by genes located on the region of difference 1 (RD1).[41] These genes are not shared by Bacille-Calmette Guerin (BCG) strains and most non-tuberculous mycobacteria (except *M. kansasii*, *M. szulgai*, and *M. marinum*).[42] These antigens are highly specific indicators of *M. tuberculosis* complex infection, and

have been shown to elicit strong IFN-γ responses from the T cells of persons infected with *M. tuberculosis*.[43] Immune responses to these and several other antigens have been studied in sarcoidosis.

T-cell Responses

Sarcoidosis is characterised by polarised CD4+ T cells with a Th1 immunophenotype.[44] Dubaniewicz, *et al* studied *M. tuberculosis*-heat shock protein stimulated T-cell subsets and Th1/Th2 cytokine patterns in the peripheral blood mononuclear cell cultures from 22 sarcoidosis patients, 20 TB patients and 20 healthy controls.[44] Their results showed that mtb-hsp stimulation lead to increased levels of pro-inflammatory cytokines, TNF-α, and IL-6 in sera from sarcoidosis and TB patients in comparison with healthy controls. Moreover, sarcoidosis patients demonstrated the lowest levels of IL-4 and the highest levels of IL-10. Two different studies[45,46], identified Th1 immune responses in peripheral blood mononuclear cells (PBMCs) to ESAT-6, katG and superoxide dismutase (SodA) in 20/56 (*versus* 2/50 purified protein derivative [PPD] negative controls), 22/56 (*versus* 0/50 PPD negative controls) and 12/30 (*versus* 0/26 PPD negative controls), respectively in patients with sarcoidosis. These studies[45,46] suggested that the sarcoidosis immune response may be against mycobacterial virulence factors. This was further studied by cellular recognition patterns against virulence factors, such as antigen 85A (Ag85A), that can differentiate mycobacterial species.[47] In another study, T-cell responses to Ag85A (mycolyl transferase) were found in 15 of 25 patients with sarcoidosis as against 2 of 22 PPD negative controls.[48] Immune responses against Ag85A in sarcoidosis have confirmed that similar to patients with mycobacterial infections such as TB or leprosy, sarcoidosis patients recognised multiple distinct epitopes of Ag85A. However, Ag85A peptides recognised by sarcoidosis patients were distinct from the Ag85A peptides recognised by patients infected with *M. tuberculosis* or *M. leprae*. Another mycobacterial virulence factor, sodA, has been isolated from sarcoidosis specimens and its characterisation by molecular techniques have demonstrated nucleic acid sequences closest to *M. tuberculosis*, yet distinct. Further, peptides translated from these sequences has been shown to evoke Th-1 immunophenotype in the sarcoidosis PBMCs.[49]

T-cell immune responses against *M. tuberculosis* antigens have also been studied in BAL in sarcoidosis. Although the Th1 immune responses were present systemically, it was shown that katG-reactive CD4+ Th1 cells preferentially accumulated in the lung, indicating a compartmentalised response.[50] This study also demonstrated that circulating katG-reactive T cells were found in chronic active sarcoidosis but not in patients with inactive disease. Further, the responses were similar in patients with or without Lofgren's syndrome, and were not influenced by phenotypic, genetic, or prognostic characteristics. Similar loss of immune responses to mycobacterial virulence factors after resolution of TB has also been observed.[51] More recently, another study on diagnostic BAL in sarcoidosis[52] demonstrated similar compartmentalised immune responses, and that induction of innate immunity by toll-like receptor 2 contributes to the polarised Th1 immune response. Recognition was significantly absent from BAL fluid cells from patients with other lung diseases, including infectious granulomatous diseases.

The detection of T-cell responses against ESAT-6, katG, and SodA provides a mechanism for more in-depth analysis of pathogenesis of sarcoidosis. It suggests exposure of sarcoidosis patients to pathogenic mycobacterial species. These proteins are typically secreted during the stage of active mycobacterial replication, compared with expression of other proteins that are expressed when mycobacteria are in the latent state.[53] However, a recent study from Germany[54] failed to replicate these findings. It was observed that IFN-γ production in response to *ex vivo* contact with PPD, ESAT-6 or CFP-10 by mononuclear cells in the BAL or peripheral blood was comparable among patients with sarcoidosis and controls, but was less frequently observed in both groups compared to patients with TB. It has also been shown that mycobacterial ESAT-6 and katG are recognised by sarcoidosis CD4+ T cells when presented by known sarcoidosis susceptibility allele, DRB1*1101.[55] It is possible that the presence of mycobacterial infection or BCG vaccination in genetically predisposed host may be involved in the development of autoimmunity.[56]

Tuberculin Anergy and Interferon-Gamma Release Assays

Another clinically important phenomenon that occurs in sarcoidosis is the depression of delayed type hypersensitivity. Commonly utilised as a diagnostic tool, this is often impaired in active sarcoidosis and not seen when sarcoidosis resolves. Various mechanisms have been postulated to explain this phenomenon. In one of the earlier studies,[57] it was shown that the T-lymphocytes from blood and BAL of sarcoidosis patients had reduced proliferative responses to various antigens, such as PPD, *Candida* and tetanus. Similar weaker responses to PPD were demonstrated in patients with ocular sarcoidosis from Japan.[58] A subgroup of CD4+ T cells may account for this anergy by abolishing the IL-2 production and inhibiting the T-cell proliferation.[59,60] It is also suggested that the anergy state may be

related to diminished dendritic cell function.[61] Anergy to tuberculin skin test (TST) in sarcoidosis has also been well described from India. In our earlier study,[62] despite the high prevalence of latent TB infection (LTBI) in the population, most of the patients (95%) with sarcoidosis were 'TST negative' with 1 tuberculin units (TUs) PPD using a cut-off >10mm. A negative TST with a cut-off of 10mm reaction to 5 TU PPD had virtually 100% sensitivity for a diagnosis of sarcoidosis, making TST an important test in the diagnostic work-up of sarcoidosis. Tuberculin testing is widely applied in sarcoidosis, primarily to rule out TB as a differential diagnosis in countries with high prevalence of TB and to rule out LTBI prior to initiating these patients on therapies known to cause increased risk of reactivation of TB, such as TNF-α inhibitors.

Interferon-gamma release assays (IGRAs) have several advantages over the TST and are superior to TST in several groups of healthy individuals.[63-65] As the test is done *in vitro* and does not involve measurements such as skin induration, the results are less subjective, and a single visit by the patient is sufficient. Newer, RD1-based IGRAs are also thought to be more sensitive and specific than the PPD-based TST. The test is based on the principle that T cells from a whole blood sample, when exposed and incubated with a specific *M. tuberculosis* antigen (ESAT-6, CFP-10) will produce IFN- γ. These proteins are absent from all BCG strains and from most non-tuberculosis mycobacteria, making these tests very specific for *M. tuberculosis*. Carlisle and colleagues,[46] found a significant difference among the sarcoidosis and tuberculin negative controls to ESAT-6. Similarly, Drake and colleagues[45] detected Th1 immune responses to *M. tuberculosis* ESAT-6 and KatG peptides from peripheral blood mononuclear cells in sarcoidosis but peripheral anergy to PPD. In contrast, the responses of Japanese patients with sarcoidosis to QuantiFERON-TB Gold (QFT) using ESAT-6 and CFP-10 showed positivity rate of QuantiFERON-TB Gold in only 3.3 percent.[66] The differing responses to ESAT-6 and CFP-10 as measured by the two IGRAs used in Japanese study and previous study by Drake *et al*[45] may reflect the differing methodology used in the two tests.[67] In a recent study,[68] we have shown that there is high positivity of QFT in patients with sarcoidosis, which is similar to the positivity in healthy volunteers, reflecting the high population prevalence of LTBI in our country. In clinical practice, we have often observed that a diagnosis of TB in patients with sarcoidosis is made based on a positive QFT. Our studies have shown that there is an acquired tuberculin anergy in patients of sarcoidosis; however results of QFT are not similarly affected. The QFT being more sensitive test than the TST continues to remain positive in many patients with sarcoidosis, and thus, may be more accurate to detect LTBI in these patients. Also, in high TB prevalence countries, a negative TST had a better value in the diagnosis of sarcoidosis and a positive QFT should not be considered to rule out sarcoidosis.

B-cell Responses

Clear elucidation of the appropriate antigen and humoral responses to them in cohorts of TB and sarcoidosis patients promises a novel approach to study the relationship between these two granulomatous pathologies. Few investigators have reported on the detection and humoral response of sarcoidosis patients to *M. tuberculosis* antigens. In one of the earliest reports, Levy *et al*[68] had reported that sarcoidosis patients showed lower values of mean optical density of serum immunoglobulin (Ig)-G using adsorbed mycobacterial sonicates as antigens when compared to active TB. Song *et al*[22] found anti-mycobacterial katG IgG in the sera of 12 of 25 (48%) patients with sarcoidosis subjects compared with 0 of 11 PPD-negative healthy controls.[22] Using matrix-assisted laser desorption ionisation time-off flight (MALDI-TOF) mass spectrometry, they identified peptide sequences that corresponded to *M. tuberculosis* katG and one potential match for *M. tuberculosis* topoisomerase. In a separate sarcoidosis specimen, they found four of 26 peptides that corresponded to the katG of *M. smegmatis*.[22] Moller[69] also reported that myco-bacterial KatG was detectable in Kviem's reagent and that IgG responses to recombinant mKatG was detectable in more than 50% of patients with sarcoidosis but rarely in PPD-negative controls. In another study of immune responses to mycobacterial proteins among patients with sarcoidosis, TB and BCG-vaccinated controls; Dubaniewicz and colleagues[70] reported that 12 of 37 patients with sarcoidosis demonstrated a humoral response to *M. Tuberculosis*-heat shock protein 70 (hsp70), compared with none of the 18 controls, and six of 29 TB patients.

The humoral response in sarcoidosis against RD1 antigens of *M. tuberculosis* has rarely been studied despite numerous studies of T-cell responses to RD1 antigens. There are several drawbacks of using IGRAs in developing countries to assess the relationship between TB and sarcoidosis, and distinguishing between them based on these responses. These include the high-cost, necessity of technical expertise, utilisation/separation of a living cell, and lack of widespread availability given that LTBI is not treated in the tropics. Besides, serologic responses to ESAT-6 as evidenced by antibody levels and detection of antigens may reflect a qualitatively different aspect of the immunopathogenesis in sarcoidosis as opposed to IGRAs. We evaluated the antibodies against RD1 (ESAT-6 and CFP-10) antigens in serum of patients

with sarcoidosis and demonstrated a high prevalence of antibodies against RD1 antigens in patients with sarcoidosis. The overall reactivity for any antigen was seen in 44.4% sarcoidosis samples and if only PPD-patients were used as controls, the positivity rose to 61 percent. None of the PPD positive cases were positive either for ESAT-6 or CFP-10 antibodies by the PPD negative cut-off.[71]

IMPLICATIONS AND FUTURE DIRECTIONS

Pooled data from various studies discussed above does provide a suggestive, though not unequivocal evidence in favour of mycobacteria as a cause of sarcoidosis (Table and Figures 1 and 2). Studies on the recognition of mycobacterial antigens and host T-cell and B-cell responses to them have generated many interesting hypotheses in understanding the complex immunopathogenesis of sarcoidosis and the role of mycobacteria in them. So much so, in a recent computer simulation study,[71] it was suggested that Lofgren's syndrome represents the hyper-reactive end of a spectrum of granulomatous responses to specific mycobacteria, whereas pulmonary TB and atypical mycobacterial infections might represent the opposite end. Similarly, du Bois *et al*[4] hypothesised that analogous to leprosy wherein tuberculoid (pauci-bacillary) and lepromatous (exuberant bacilli) forms represent different levels of host immune responses to the pathogen; sarcoidosis might represent the tuberculoid form of the pathological responses to mycobacteria.

There have been counter arguments to the role of mycobacteria in pathogenesis of sarcoidosis and the major points among them are: (1) *Mycobacteria* spp have never been cultured from sarcoidosis lesions; (2) the absence of nucleic acid in 50% of patients; and (3) the absence of a response to anti-tuberculosis treatment together with tuberculin anergy. However, studies do suggest that mycobacteria are a trigger, if

Table. Summary of major studies on mycobacterial antigens in sarcoidosis

Author (year)	Experimental Details	Positivity in Sarcoidosis % (n/N)	Positivity in Controls		Significance
			PPD+/Tuberculosis % (n/N)	PPD- % (n/N)	
T-cell responses					
Nishino *et al* (2000)[58]	IFN-γ by PBMCs in response to PPD	14	10	-	Weaker but similar responses to controls
Carlisle *et al* (2007)[46]	Th1 immune responses in PBMCs to: (a) ESAT-6; (b) katG; (c) SodA	(a) 40 (12/30) (b) 30 (9/30) (c) 40 (12/30)	(a) 60 (6/10)** (b) 50 (5/10)** (c) 60 (6/10)**	(a) 3.8 (1/26)* (b) 0 (0/26)* (c) 0 (0/26)*	*(a) P=0.0014; *(b) and (c) P= 0.002; ** NS
Drake *et al* (2008)[45]	Th1 immune responses in PBMCs to: ESAT-6, mkatG	57.7 (15/26)	87.5 (7/8)**	4.2 (1/24)*	* P<0.001; **P=0.21
Inui *et al* (2007)[66]	IGRA in response to ESAT-6 and CFP-10 (QFT) in blood	3.33 (3/90)	–	–	Low prevalence of T-cell response
Dubaniewicz *et al* (2007)[44]	T-cell subsets and Th1/Th2 cytokine patterns in response to Mtb-hsp	22	20	20	Increased IL-10 and decreased IL-4 in sarcoidosis
Chen *et al* (2008)[50]	T-cell responses to mKatG in PBMCs and BALMCs	Higher median spot forming cells			Higher as compared to PPD-controls and higher in BAL as compared to PBMCs
Horster *et al* (2009)[54]	IGRA in response to ESAT-6 and CFP-10 by: (a) PBMCs; (b) BALMCs	(a) 29.4 (5/17) (b) 46.7 (7/15)	(a) 93.9 (31/33)** (b) 84.4 (27/32)**	(a) 48.6 (17/35)* (b) 24 (7/29)*	*(a) P=0.23; *(b) P=0.17; ** (a) P< 0.05; **(b) P<0.001
Oswald-Richter *et al* (2009)[52]	T-cell responses in BAL to: ESAT-6, mkatG	72.7 (32/44)	–	3.7 (1/27)*	* P<0.001
Gupta *et al* (2010)[68]	IGRA in response to ESAT-6 and CFP-10 (QFT) in blood	30 (9/30)	80.9 (9/11)**	30.1 (5/16)	* P=0.03; ** P=0.009
B-cell responses					
Levy *et al* (1988)[69]	Anti TB IgG by ELISA to adsorbed mycobacterial sonicates	11	7	7	TB patients had significantly higher mean OD for IgG compared to sarcoidosis or controls
Song *et al* (2005)[22]	IgG antibodies to recombinant mKatG	48 (12/45)	40 (4/10)**	0 (0/11)*	*P=0.005; **P=0.72
Dubaniewicz *et al* (2006)[71]	Serum anti-Mtb-hsp70 antibodies	32.4 (12/37)	20.6 (2/29)**	0 (0/18)*	*P=.000; **P=0.43
Hajizadeh *et al* (2007)[48]	Recognition of mycobacterial antigen 85A by the PBMCs	60 (15/25)	87.5 (14/16)**	9.1 (2/22)*	*P=0.0006; **P=0.08
Agarwal *et al* (2009)[72]	Antibodies against RD1 (ESAT-6 and CFP-10) antigens in serum	61.1 (11/18)	90 (9/10)**	0 (0/20)*	*P=0.0002; **P=0.24

PPD=Purified protein derivative, IFN-γ=Interferon-gamma, PBMCs=Peripheral blood monocytes, ESAT-6=Early secretory antigen-6, SodA=Superoxide dismutase A, mkatG=Mycobacterial catalase-peroxidase, IGRAs=Interferon-gamma release assays, CFP-10=Culture filtrate protein-10, QFT=Quanti FERON-TB, IL=Interleukin, BALMCs=Bronchoalveolar lavage monocytes, BAL=Bronchoalveolar lavage, TB=Tuberculosis, IgG=Immunoglobulin G, ELISA=Enzyme linked immunosorbent assay, OD=Optial density, Mtb-hsp70=*Mycobacterium tuberculosis*-heat shock protein 70, RD1=Region of differentiation 1

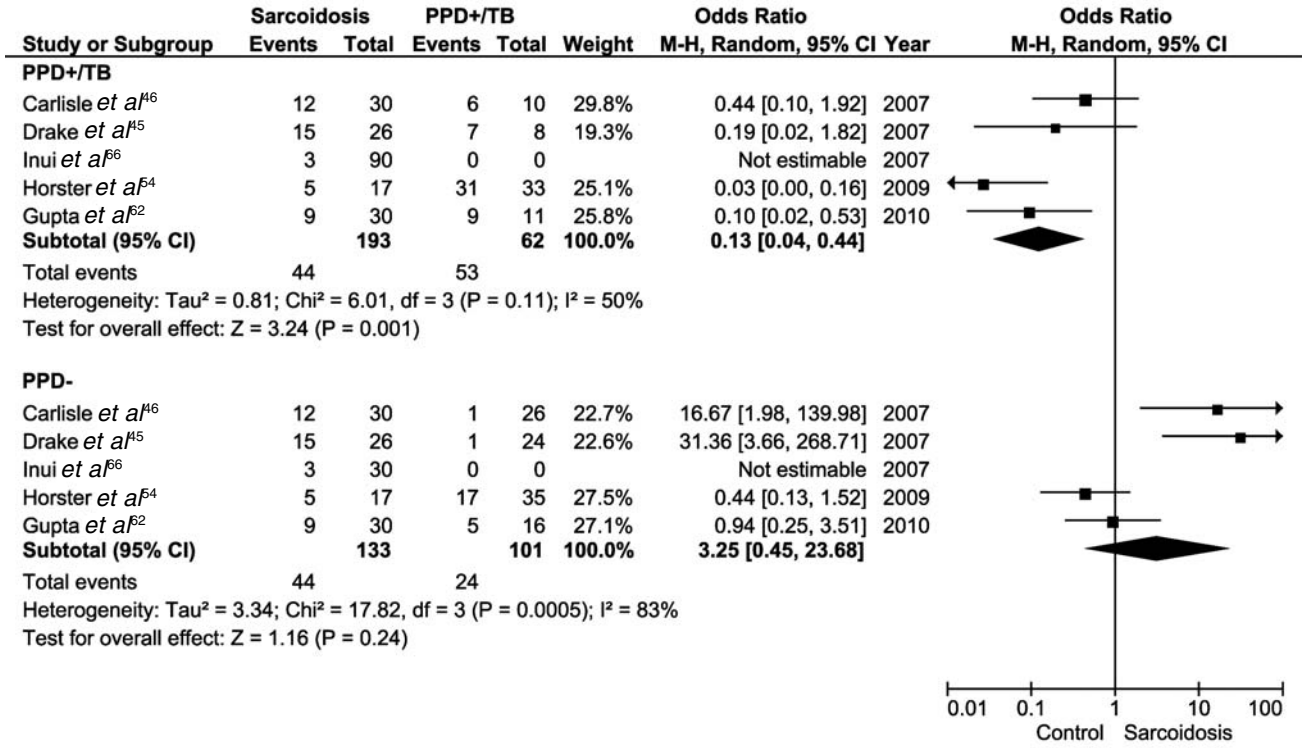

Figure 1. Forest plot (odds ratio [OR], 95% confidence interval [CI]) displaying the studies that have assessed T-cell responses against RD1 antigens (ESAT-6, CFP-10) in patients with sarcoidosis compared to PPD+/TB patients and PPD- controls. The plot shows that T-cell responses are significantly demonstrable in PPD+/TB compared to sarcoidosis and there is a trend towards higher responses in sarcoidosis as compared to PPD- controls (random effects model).

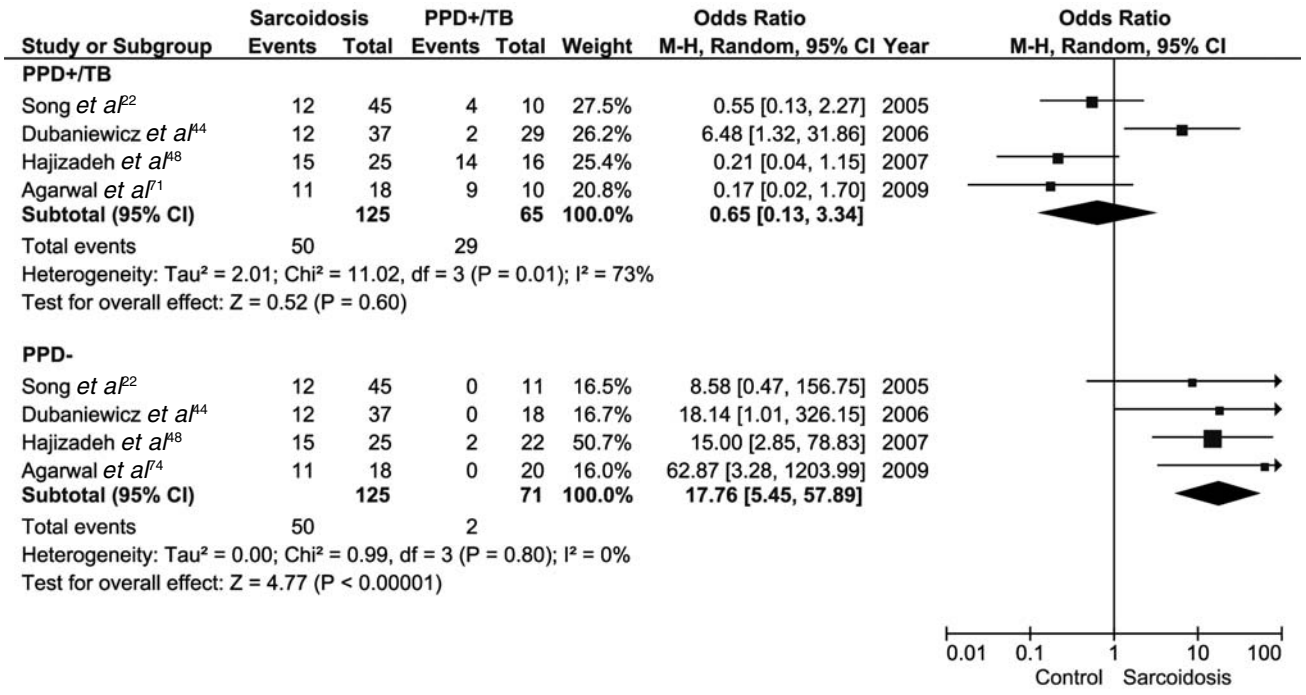

Figure 2. Forest plot (odds ratio [OR], 95% confidence interval [CI]) displaying the studies that have assessed B-cell responses against specific mycobacterial proteins (ESAT-6, CFP-10, katG, sodA) in patients with sarcoidosis compared to PPD+/TB patients and PPD- controls. The plot shows that humoral responses are significantly demonstrable in sarcoidosis compared to PPD- controls and are similar in sarcoidosis compared to PPD+/TB subjects (random effects model).

not an infection, and therefore there may not necessarily be a response to anti-tubercular therapy.

Further research, in addition to identifying microbial species that may have a role in the pathogenesis of sarcoidosis, should also focus on identification of microbial proteins that contribute to sarcoidosis resolution or disease progression. Future molecular efforts should delineate whether the nucleic acids or proteins detected reflect actively replicating organisms or persistent proteins. Focus on other microbial virulence factors would also provide greater insight into pathogenic mechanisms of sarcoidosis and also identify likely therapeutic targets.

To conclude, even if there is no clear evidence to support the contention that tubercle bacillus causes sarcoidosis, the various studies discussed above reinforce the possible pathogenic role of mycobacterial antigens in sarcoidosis. At the same time, these also limits the clinical value of the molecular and serological studies based on mycobacterial antigens in the differential diagnosis of TB from sarcoidosis, particularly in a country like India with high endemicity for TB.

REFERENCES

1. Perez RL, Rivera-Marrero CA, Roman J. Pulmonary granulomatous inflammation: from sarcoidosis to tuberculosis. *Semin Respir Infect* 2003;18:23-32.
2. Newman LS. Aetiologies of sarcoidosis. *Eur Respir Mon* 2005;32:23-48.
3. Thomeer M, Demedts M, Wuyts W. Epidemiology of sarcoidosis. *Eur Respir Mon* 2005;32:13-22.
4. du Bois RM, Goh N, McGrath D, Cullinan P. Is there a role for microorganisms in the pathogenesis of sarcoidosis? *J Intern Med* 2003;253:4-17.
5. Vidal S, de la Horra C, Martin J, Montes-Cano MA, Rodriguez E, Respaldiza N, *et al*. *Pneumocystis jirovecii* colonisation in patients with interstitial lung disease. *Clin Microbiol Infect* 2006;12:231-5.
6. Drake WP, Newman LS. Mycobacterial antigens may be important in sarcoidosis pathogenesis. *Curr Opin Pulm Med* 2006;12:359-63.
7. Ishige I, Eishi Y, Takemura T, Kobayashi I, Nakata K, Tanaka I, *et al*. Propionibacterium acnes is the most common bacterium commensal in peripheral lung tissue and mediastinal lymph nodes from subjects without sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis* 2005;22:33-42.
8. Oswald-Richter KA, Drake WP. The etiologic role of infectious antigens in sarcoidosis pathogenesis. *Semin Respir Crit Care Med* 2010;31:375-9.
9. Sharma OP, Murray Kornfeld, American College of Chest Physicians, and sarcoidosis: a historical footnote: 2004 Murray Kornfeld Memorial Founders Lecture. *Chest* 2005; 128:1830-5.
10. Gupta D, Agarwal R, Aggarwal AN, Jindal SK. Molecular evidence for the role of mycobacteria in sarcoidosis: a meta-analysis. *Eur Respir J* 2007;30:508-16.
11. Mootha VK, Agarwal R, Aggarwal AN, Gupta D, Ahmed J, Verma I, *et al*. The sarcoid-tuberculosis link: evidence from a high TB prevalence country. *J Infect* 2010; 60:501-3.
12. Kent DC, Houk VN, Elliott RC, Sokolowski JW Jr, Baker JH, Sorensen K. The definitive evaluation of sarcoidosis. *Am Rev Respir Dis* 1970;101:721-7.
13. Hatzakis K, Siafakas NM, Bouros D. Miliary sarcoidosis following miliary tuberculosis. *Respiration* 2000;67:219-22.
14. Vanek J, Schwarz J. Demonstration of acid-fast rods in sarcoidosis. *Am Rev Respir Dis* 1970;101:395-400.
15. Cantwell AR Jr. Variably acid-fast bacteria in a case of systemic sarcoidosis and hypodermitis sclerodermiformis. *Dermatologica* 1981;163:239-48.
16. Cantwell AR Jr. Histologic observations of variably acid-fast pleomorphic bacteria in systemic sarcoidosis: a report of 3 cases. *Growth* 1982;46:113-25.
17. Mitchell DN, Rees RJ. A transmissible agent from sarcoid tissue. *Lancet* 1969;2:81-4.
18. Mitchell DN, Rees RJ. An attempt to demonstrate a transmissible agent from sarcoid material. *Postgrad Med J* 1970;46:510-4.
19. Mitchell DN, Rees RJ, Goswami KK. Transmissible agents from human sarcoid and Crohn's disease tissues. *Lancet* 1976;2:761-5.
20. Mitchell DN. The nature and physical characteristics of a transmissible agent from human sarcoid tissue. *Ann N Y Acad Sci* 1976;278:233-48.
21. Almenoff PL, Johnson A, Lesser M, Mattman LH. Growth of acid fast L forms from the blood of patients with sarcoidosis. *Thorax* 1996;51:530-3.
22. Song Z, Marzilli L, Greenlee BM, Chen ES, Silver RF, Askin FB, *et al*. Mycobacterial catalase-peroxidase is a tissue antigen and target of the adaptive immune response in systemic sarcoidosis. *J Expir Med* 2005;201:755-67.
23. Rybicki BA, Hirst K, Iyengar SK, Barnard JG, Judson MA, Rose CS, *et al*. A sarcoidosis genetic linkage consortium: the sarcoidosis genetic analysis (SAGA) study. *Sarcoidosis Vasc Diffuse Lung Dis* 2005;22:115-22.
24. Schurmann M. Genetics of sarcoidosis. *Semin Respir Crit Care Med* 2003;24:213-22.
25. Hunninghake GW, Crystal RG. Pulmonary sarcoidosis: a disorder mediated by excess helper T-lymphocyte activity at sites of disease activity. *N Engl J Med* 1981;305:429-34.
26. Semenzato G, Pezzutto A, Chilosi M, Pizzolo G. Redistribution of T lymphocytes in the lymph nodes of patients with sarcoidosis. *N Engl J Med* 1982;306:48-9.
27. Grunewald J, Eklund A. Role of CD4+ T cells in sarcoidosis. *Proc Am Thorac Soc* 2007;4:461-4.
28. Silver RF, Crystal RG, Moller DR. Limited heterogeneity of biased T-cell receptor V beta gene usage in lung but not blood T cells in active pulmonary sarcoidosis. *Immunology* 1996;88:516-23.
29. Konishi K, Moller DR, Saltini C, Kirby M, Crystal RG. Spontaneous expression of the interleukin 2 receptor gene and presence of functional interleukin 2 receptors on T lymphocytes in the blood of individuals with active pulmonary sarcoidosis. *J Clin Invest* 1988;82:775-81.
30. Robinson BW, McLemore TL, Crystal RG. Gamma interferon is spontaneously released by alveolar macrophages and lung T lymphocytes in patients with pulmonary sarcoidosis. *J Clin Invest* 1985;75:1488-95.
31. Baughman RP, Strohofer SA, Buchsbaum J, Lower EE. Release of tumor necrosis factor by alveolar macrophages of patients with sarcoidosis. *J Lab Clin Med* 1990;115:36-42.
32. Moller DR, Forman JD, Liu MC, Noble PW, Greenlee BM, Vyas P, *et al*. Enhanced expression of IL-12 associated with Th1 cytokine profiles in active pulmonary sarcoidosis. *J Immunol* 1996;156:4952-60.
33. Agostini C, Trentin L, Facco M, Sancetta R, Cerutti A, Tassinari C, *et al*. Role of IL-15, IL-2, and their receptors in the development of T cell alveolitis in pulmonary sarcoidosis. *J Immunol* 1996;157:910-8.
34. Kreipe H, Radzun HJ, Heidorn K, Barth J, Kiemle-Kallee J, Petermann W, *et al*. Proliferation, macrophage colony-

stimulating factor, and macrophage colony-stimulating factor-receptor expression of alveolar macrophages in active sarcoidosis. *Lab Invest* 1990;62:697-703.
35. Pinkston P, Bitterman PB, Crystal RG. Spontaneous release of interleukin-2 by lung T lymphocytes in active pulmonary sarcoidosis. *N Engl J Med* 1983;308:793-800.
36. Hunninghake GW, Bedell GN, Zavala DC, Monick M, Brady M. Role of interleukin-2 release by lung T-cells in active pulmonary sarcoidosis. *Am Rev Respir Dis* 1983;128:634-8.
37. Agostini C, Adami F, Semenzato G. New pathogenetic insights into the sarcoid granuloma. *Curr Opin Rheumatol* 2000;12:71-6.
38. Girgis RE, Basha MA, Maliarik M, Popovich J Jr, Iannuzzi MC. Cytokines in the bronchoalveolar lavage fluid of patients with active pulmonary sarcoidosis. *Am J Respir Crit Care Med* 1995;152:71-5.
39. Shigehara K, Shijubo N, Ohmichi M, Takahashi R, Kon S, Okamura H, *et al*. IL-12 and IL-18 are increased and stimulate IFN-gamma production in sarcoid lungs. *J Immunol* 2001;166:642-9.
40. Bingisser R, Speich R, Zollinger A, Russi E, Frei K. Interleukin-10 secretion by alveolar macrophages and monocytes in sarcoidosis. *Respiration* 2000;67:280-6.
41. Berthet FX, Rasmussen PB, Rosenkrands I, Andersen P, Gicquel B. A Mycobacterium tuberculosis operon encoding ESAT-6 and a novel low-molecular-mass culture filtrate protein (CFP-10). *Microbiology* 1998;144:3195-203.
42. Lalvani A, Nagvenkar P, Udwadia Z, Pathan AA, Wilkinson KA, Shastri JS, *et al*. Enumeration of T cells specific for RD1-encoded antigens suggests a high prevalence of latent Mycobacterium tuberculosis infection in healthy urban Indians. *J Infect Dis* 2001;183:469-77.
43. Kunst H. Diagnosis of latent tuberculosis infection: the potential role of new technologies. *Respir Med* 2006; 100:2098-106.
44. Dubaniewicz A, Trzonkowski P, Dubaniewicz-Wybieralska M, Singh M, Mysliwski A. Mycobacterial heat shock protein-induced blood T lymphocytes subsets and cytokine pattern: comparison of sarcoidosis with tuberculosis and healthy controls. *Respirology* 2007;12:346-54.
45. Drake WP, Dhason MS, Nadaf M, Shepherd BE, Vadivelu S, Hajizadeh R, *et al*. Cellular recognition of Mycobacterium tuberculosis ESAT-6 and KatG peptides in systemic sarcoidosis. *Infect Immun* 2007;75:527-30.
46. Carlisle J, Evans W, Hajizadeh R, Nadaf M, Shepherd B, Ott RD, *et al*. Multiple Mycobacterium antigens induce interferon-gamma production from sarcoidosis peripheral blood mononuclear cells. *Clin Exp Immunol* 2007;150:460-8.
47. Launois P, DeLeys R, Niang MN, Drowart A, Andrien M, Dierckx P, *et al*. T-cell-epitope mapping of the major secreted mycobacterial antigen Ag85A in tuberculosis and leprosy. *Infect Immun* 1994;62:3679-87.
48. Hajizadeh R, Sato H, Carlisle J, Nadaf MT, Evans W, Shepherd BE, *et al*. Mycobacterium tuberculosis antigen 85A induces Th-1 immune responses in systemic sarcoidosis. *J Clin Immunol* 2007;27:445-54.
49. Allen SS, Evans W, Carlisle J, Hajizadeh R, Nadaf M, Shepherd BE, *et al*. Superoxide dismutase A antigens derived from molecular analysis of sarcoidosis granulomas elicit systemic Th-1 immune responses. *Respir Res* 2008;9:36.
50. Chen ES, Wahlstrom J, Song Z, Willett MH, Wiken M, Yung RC, *et al*. T cell responses to mycobacterial catalase-peroxidase profile a pathogenic antigen in systemic sarcoidosis. *J Immunol* 2008;181:8784-96.
51. Pathan AA, Wilkinson KA, Klenerman P, McShane H, Davidson RN, Pasvol G, *et al*. Direct ex vivo analysis of antigen-specific IFN-gamma-secreting CD4 T cells in Mycobacterium tuberculosis-infected individuals: associations with clinical disease state and effect of treatment. *J Immunol* 2001;167:5217-25.
52. Oswald-Richter KA, Culver DA, Hawkins C, Hajizadeh R, Abraham S, Shepherd BE, *et al*. Cellular responses to mycobacterial antigens are present in bronchoalveolar lavage fluid used in the diagnosis of sarcoidosis. *Infect Immun* 2009;77:3740-8.
53. Andersen P. The T cell response to secreted antigens of Mycobacterium tuberculosis. *Immunobiology* 1994; 191: 537-47.
54. Horster R, Kirsten D, Gaede KI, Jafari C, Strassburg A, Greinert U, *et al*. Antimycobacterial immune responses in patients with pulmonary sarcoidosis. *Clin Respir J* 2009; 3:229-38.
55. Oswald-Richter K, Sato H, Hajizadeh R, Shepherd BE, Sidney J, Sette A, *et al*. Mycobacterial ESAT-6 and katG are recognized by sarcoidosis CD4+ T cells when presented by the American sarcoidosis susceptibility allele, DRB1*1101. *J Clin Immunol* 2010;30:157-66.
56. Dubaniewicz A. Mycobacteriumtuberculosis heat shock proteins and autoimmunity in sarcoidosis. *Autoimmun Rev* 2010;9:419-24.
57. Lecossier D, Valeyre D, Loiseau A, Cadranel J, Tazi A, Battesti JP, *et al*. Antigen-induced proliferative response of lavage and blood T lymphocytes: comparison of cells from normal subjects and patients with sarcoidosis. *Am Rev Respir Dis* 1991;144:861-8.
58. Nishino K, Yoshida H, Yoshida O, Watanabe M, Fukushima A, Ueno H. Analysis of responses of peripheral blood lymphocytes from sarcoidosis patients to purified protein derivative. *Jpn J Ophthalmol* 2000;44:165-70.
59. Miyara M, Amoura Z, Parizot C, Badoual C, Dorgham K, Trad S, *et al*. The immune paradox of sarcoidosis and regulatory T cells. *J Expir Med* 2006;203:359-70.
60. Hudspith BN, Flint KC, Geraint-James D, Brostoff J, Johnson NM. Lack of immune deficiency in sarcoidosis: compartmentalisation of the immune response. *Thorax* 1987;42:250-5.
61. Mathew S, Bauer KL, Fischoeder A, Bhardwaj N, Oliver SJ. The anergic state in sarcoidosis is associated with diminished dendritic cell function. *J Immunol* 2008;181:746-55.
62. Gupta D, Chetty M, Kumar N, Aggarwal AN, Jindal SK. Anergy to tuberculin in sarcoidosis is not influenced by high prevalence of tuberculin sensitivity in the population. *Sarcoidosis Vasc Diffuse Lung Dis* 2003;20:40-5.
63. Hardy AB, Varma R, Collyns T, Moffitt SJ, Mullarkey C, Watson JP. Cost-effectiveness of the NICE guidelines for screening for latent tuberculosis infection: the QuantiFERON-TB Gold IGRA alone is more cost-effective for immigrants from high burden countries. *Thorax* 2010; 65:178-80.
64. Winje BA, Oftung F, Korsvold GE, Mannsaker T, Ly IN, Harstad I, *et al*. School based screening for tuberculosis infection in Norway: comparison of positive tuberculin skin test with interferon-gamma release assay. *BMC Infect Dis* 2008;8:140.
65. Lee SS, Liu YC, Huang TS, Chen YS, Tsai HC, Wann SR, *et al*. Comparison of the interferon-gamma release assay and the tuberculin skin test for contact investigation of tuberculosis in BCG-vaccinated health care workers. *Scand J Infect Dis* 2008;40:373-80.
66. Inui N, Suda T, Chida K. Use of the QuantiFERON-TB Gold test in Japanese patients with sarcoidosis. *Respir Med* 2008;102:313-5.
67. Ferrara G, Losi M, D'Amico R. Use in routine clinical practice of two commercial blood tests for diagnosis of infection with Mycobacterium tuberculosis: a prospective study. *Lancet* 2006;367:1328-34.

68. Gupta D, Kumar S, Verma I, Agarwal R. Interferon gamma release assay (IGRA) in sarcoidosis patients from a high tuberculosis (TB) prevalence country. *Am J Respir Crit Care Med* 2010;181:A2366.
69. Levy H, Feldman C, Wadee AA, Rabson AR. Differentiation of sarcoidosis from tuberculosis using an enzyme-linked immunosorbent assay for the detection of antibodies against Mycobacterium tuberculosis. *Chest* 1988;94:1254-5.
70. Moller DR. Potential etiologic agents in sarcoidosis. *Proc Am Thorac Soc* 2007;4:465-8.
71. Dubaniewicz A, Kampfer S, Singh M. Serum anti-mycobacterial heat shock proteins antibodies in sarcoidosis and tuberculosis. *Tuberculosis (Edin)* 2006;86:60-7.
72. Agarwal R, Gupta D, Srinivas R, Verma I. Role of proteins encoded by RD1 of Mycobacterium tuberculosis in differentiating tuberculosis (TB) from sarcoidosis in a high TB prevalence country. *Chest* 2009;136:44S-f-
73. Saltini C, Pallante M, Puxeddu E, Contini S, Voorter CE, Drent M, *et al*. M. avium binding to HLA-DR expressed alleles in silico: a model of phenotypic susceptibility to sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis* 2008;25:100-16.

Full text articles published in IJCDAS from July-September 2003 onwards can be accessed online on Internet through the following sites

V.P. Chest Institute's site: http:// www.vpci.org.in

Indmed's site: http://medind.nic.in

Guidance for Authors appears in every issue.

Authors' Index appears in the last issue of the year

Clinical Trials Registry-India

A Clinical Trials Registry-India has been set up jointly by the Department of Science and Technology (DST), World Health Organisation (WHO) and Indian Council of Medical Research at the National Institute of Medical Statistics (NIMS), New Delhi. This Registry will provide a platform for registration of all clinical trials. The objective of the Registry is to establish a public record system by registering all prospective clinical trials of any intervention (drug, surgical procedure, preventive measures, lifestyle modifications, devices, educational or behavioural treatment, rehabilitation strategies and complementary therapies) conducted in India involving human participants. The Registry will be made publicly available on the internet at no cost. The website of the Indian Registry is www.ctri.in.