Original article

Prospective study of immunomodulation in osteoarticular tuberculosis non responsive to anti tubercular therapy

Saurabh*, B.P. Sharma, Amit Kumar, Saubhik Das, Shambhu Prasad

Dept of Orthopaedics, Central Institute of Orthopaedics (CIO), Vardhman Mahavir Medical College and Safdarjung Hospital, Delhi, India

ARTICLE INFO

Article history:

Received 4 October 2016

Received in revised form 22 May 2017

Accepted 27 June 2017

Available online 3 July 2017

Keywords:

Osteoarticular tuberculosis

Immunomodulation

Levamisole

CD4

CD8

ABSTRACT

Background: Tuberculosis is one of the common disease of Indian subcontinent. India has the highest TB burden, accounting for one fifth of the global incidence. Previous studies have shown positive response of immunomodulation in patients of osteoarticular tuberculosis. We did this prospective study to assess the role of immunomodulation in the cases of osteoarticular tuberculosis who were not responding to anti tubercular therapy.

Materials and method: This study was conducted in our institute. New patients presenting to OPD who were diagnosed with tuberculosis with no previous history of taking anti tubercular treatment were enrolled. Total of 109 patients were diagnosed clinicoradiologically. Four patients, where diagnosis was not confirmed, histopathological examination of tissue was done and the diagnosis was confirmed as tubercular in origin. Newly diagnosed patients were put on anti tubercular therapy (ATT) after documenting their blood parameters (complete hemogram, ESR, serum proteins, CD4 & CD8 counts). After 3 months patients were re-evaluated and favourably responding patients were labelled group A. Those who did not respond to therapy were put on immunomodulation along with continuation of ATT. These were designated as group B and blood parameters were compared with pre immunomodulation values.

Results: Out of total 109 patients registered, 73 patients were enlisted in group A, while 30 patients were found to be non-responsive to ATT and were included in non-responder group B (six lost to follow up). In group B the blood parameters after one month of completion of immunomodulation showed statistically significant increase in CD4 & CD8 counts ($p=0.04$). Mean CD4 count increased from $488/\text{mm}^3$ to $747/\text{mm}^3$ while CD8 count showed increment from $494/\text{mm}^3$ to $617/\text{mm}^3$. Mean haemoglobin values increased along with decrease in ESR and total leucocyte count. In group A mean CD4 and CD8 counts increased with ATT though the results were not significant statistically.

Conclusion: Treatment of tuberculosis has been a great success after introduction of chemotherapy specifically targeting Mycobacterium tuberculosis. In this study relationship between the increase in the peripheral T-cell count and a favourable clinical outcome was further associated with concomitant increase in the haemoglobin level as well as by a decrease in the ESR, total leucocyte count. In our study immunomodulation has shown a good promise to be of adjunctive use in patients on ATT with non-responsiveness.

© 2017

1. Introduction

Tuberculosis, one of the commonest disease of Indian subcontinent, is a much-studied topic since inception of modern era of medicine. Still osteoarticular tuberculosis (contributing 1–3% of total cases¹ of tuberculosis) has not received such attention as pulmonary tuberculosis has received. India has the highest TB

burden in world accounting for 2.2 million cases out of global annual incidence of 9.6 million TB cases.² Despite of having effective multi drug chemotherapy regimen, problem of disease becoming nonresponsive to treatment still persists. About 90% of those infected with *M. tuberculosis* have asymptomatic infection, with only a 10% lifetime chance that the latent infection will progress to overt, active tuberculous disease.³ In those with HIV, the risk of developing active TB increases to nearly 10% a year.³ If effective treatment is not given, the death rate for active TB cases is up to 66%.

* Corresponding author at: 145c, Gautam Nagar, Near Gujjar Dairy, Behind Indian Oil Building, New Delhi, 110049, India.

E-mail address: dr.saurabh.sjh@gmail.com (Saurabh).

Table 1
Group A (n = 73).

variable	Mean at first visit	Mean at second visit (3rd month)	Mean at third visit (6th month)	Statistical Significance (p < 0.05)
CD 4 Cell Count	609/mm ³	701/mm ³	645/mm ³	Not significant
CD 8 Cell Count	533/mm ³	694/mm ³	577/mm ³	Not significant
Hemoglobin	10.7 g/dl	13.4 g/dl	13.0 g/dl	significant
Total Leucocyte Count	8961/mm ³	8034/mm ³	7536/mm ³	significant
ESR	44.3 mm/h	21.75 mm/h	22.15 mm/h	significant
Total protein	6.9 g/dl	7.1 g/dl	6.9 g/dl	Not significant

Normal adult values: CD4 cells – 687 ± 219/mm³ and CD8 cells – 611 ± 288/mm³.⁹

Extrapulmonary TB occurs more commonly in immunosuppressed persons and young children.³ In those with HIV, this occurs in more than 50% of cases. Humans mount cell mediated immuneresponse to *M. tuberculosis*.^{4,5} After about 3 weeks of infection with mycobacterium tuberculosis, T_H-1 (helper T-cell) response against bacilli is mounted that activates macrophages to become bactericidal.⁶ T_H-1 cells are activated by mycobacterial antigens drained in lymph nodes, differentiation of T_H-1 cells depends on IL-12 secreted by antigen presenting cell that has encountered bacilli. Mature T_H-1 cells produce IFN-γ that drives macrophages to become competent and contain mycobacterial infection. Helper T cell play pivotal role in human immune response by activating both cell mediated and humoral immunity. Mycobacterium, like HIV targets helper t cell and overall immune response to Mtb is hence jeopardized. though human immune response to infection by tubercular bacillus is very effective due to which only about 5% of infected persons develop clinically evident disease, and only a further 5% or so develop post-primary disease later in life. The fact that a patient gets skeletal tuberculosis is a reflection of inherent poor protective response.⁷ Drugs like levamisole and vaccines like DPT and BCG have been found to have immunomodulatory properties by enhancing helper t cell number though the mechanism is still debated. We did this prospective study to assess the role of immunomodulation in the cases of osteoarticular tuberculosis who were not responding to ATT. Previous studies have showed positive response of immunomodulation in non responsive cases. This study aims to find adjunctive role of immunomodulation with ATT in patients.

2. Methods and material

This study was conducted in our institute after the clearance from institute's ethical committee. New patients presenting to OPD who were diagnosed with tuberculosis with no previous history of taking anti tubercular treatment were enrolled. Total of 109 patients were diagnosed clinicoradiologically. Four patients, where diagnosis was not confirmed, histopathological examination of

tissue was done and the diagnosis was confirmed as tubercular in origin. Newly diagnosed patients were put on anti tubercular therapy (ATT) after documenting there blood parameters (complete hemogram, ESR, serum proteins, CD4 & CD8 counts). After 3 months patients were re-evaluated and favourably responding patients were labelled group A. Those who did not respond to therapy were put on immunomodulation along with continuation of ATT. These were designated as group B and blood parameters were compared with pre immunomodulation values. Patients were followed up till 18 months after completion of treatment and data was collected on serial visits along with assessment of clinicoradiological and laboratory parameters. Newly diagnosed patients were put on first line anti tubercular therapy (ATT) consisting of

H: isoniazid – 5 to 10 mg/kg

R: rifampicin – 10 mg/kg

Z: Pyrazinamide – 20 to 35 mg/kg

E: Ethambutol – 15 to 30 mg/kg

DOTS regimen was not used rather we used protocol at our centre which involves using four drugs (rifampicin, isoniazid, pyrazinamide and ethambutol) for four months, three drugs for the subsequent three months, and two drugs (rifampicin, isoniazid) for a final 11 months. Patients with active pulmonary tuberculosis, HIV positive, having malignancy or requiring steroids for any other ailments were excluded. HIV infection is known cause of reversible decrease in CD4 and CD8 counts and hence was excluded. All the patients underwent blood investigations comprising of complete hemogram, ESR, serum proteins and CD4 CD8 counts. Liver function test was done 1 & 2 weeks after initiation of ATT and dose was adjusted in case of untoward reactions if any. After 3 months patients were re-evaluated with detailed history and examination along with fresh radiographs and investigation, those favourably responding clinicoradiologically and CD4 and CD8 counts more than 500 cells/mm³ were labelled as group A. Those who did not respond to therapy were designated as group B put on immunomodulation along with continuation of ATT. Six patients interrupted the treatment and were not followed up and their data was excluded. Abovementioned blood investigations were done at

Table 2
Group B (non responders).

Variable	Mean at first visit	Mean at second visit (3rd month)	Mean (6th month)	Statistical significance (p < 0.05)
CD 4 cell count	432/mm ³	488/mm ³	747/mm ³	significant
CD 8 cell count	458/mm ³	494/mm ³	617/mm ³	significant
Hemoglobin	10.6 g/dl	14.4 g/dl	12.0 g/dl	significant
Total Leucocyte Count	11086/mm ³	7793/mm ³	6766/mm ³	significant
ESR	43 mm/h	25 mm/h	18 mm/h	significant
Total protein	6.8 g/dl	7.1 g/dl	7.3 g/dl	Non significant

Normal adult values: CD4 cells – 687 ± 219/mm³ and CD8 cells – 611 ± 288/mm³.

Fig. 1. AP view of foot showing medial cuneiform involvement at first visit.

Fig. 2. AP view of same patient 1 month after immunomodulation.

Fig. 3. Recurring abscess after several aspirations.

the time of diagnosis & at the end of third and sixth month of treatment.

2.1. Group A (responders $n = 73$)

After 3 months of therapy seventy three patients showed clinico-radiological improvement along with CD4 and CD8 counts more than 500 cells/mm^3 , this group was designated as group A and anti-tubercular therapy was continued in this group.

2.2. Group B (non responders $n = 30$)

This group comprised patients who received anti tubercular therapy for 3 months and showed clinico radiological non responsiveness as

- Deterioration or spread of disease or appearance of additional lesions anywhere in the body with CD4 and CD8 counts $< 500 \text{ cells/mm}^3$ along with or without the following
- Non- healing or breakdown of wound (sinus/ulcer/aspiration site)
- Repeated formation of cold abscess despite repeated aspirations.

- Recurrence of a clinically healed osteoarticular tubercular lesion.
- Non improvement of constitutional symptoms, fever, appetite, pain.

In patients fulfilling any of the above criteria, immunomodulation was given as an adjunct to anti tubercular therapy regimen

2.3. Immunomodulation⁸

On first day of therapy BCG vaccination 0.1 ml was given by intradermal route and tablet levamisole (2 mg/kg body weight/day) was started for three days followed by an interval of seven days, constituting ten-day cycle of levamisole (three plus seven days). Six cycles of oral levamisole were repeated. On the 30th day of therapy (one month after the first BCG injection) injection BCG was repeated. One injection of DPT (diphtheria vaccine 2.5Lf, tetanus vaccine 5 Lf, bordetella pertusis 20,000 million per 0.5 ml) was given by intramuscular route on 60th day of therapy. Along the whole duration ATT was continued, fresh investigations were repeated one month after (sixth month completion) the DPT injection, new results were compared with pre immunomodulation values using paired *t*-test utilizing SPSS version 17.

Fig. 4. Healing of abscess once patient was put on immunomodulation.

Fig. 5. Showing a discharging sinus on lateral aspect of arm along with BCG scar.

3. Results

Out of total 109 patients registered, 79 patients were enlisted in group A (6 lost to follow-up) while 30 patients were found to be non-responsive to ATT and were included in non-responder group B (one lost to follow-up). 64 patients belonged to 15–35-year age group. Spine came out to be most common site for infection comprising 43 patients.

In group A patients, 45 were males and 28 were females. Mean CD4 and CD8 counts increased with ATT regimen though the results were not found to be statistically significant. Improvement in haemoglobin and decrease in ESR values was also noted that was statistically significant ($p=0.01$). There was clinicoradiological improvement in group A patients after 3 months of first line anti tubercular therapy. Patients reported with improvement in

appetite, reduction of pain, resolution of discharging sinuses. [Table 1](#) shows data of responder group A with non-significant increase in both CD4 & CD8 counts

In non-responders (group B) sixteen were males and fourteen were females. Thirteen patients had vertebral involvement, eight had articular involvement, two each of hand and foot, one of distal humerus and four had soft tissue involvement. Out of thirty non responders twenty three responded to adjunctive immunomodulation. Two patients with vertebral involvement were operated as their neurological status deteriorated on treatment. One patient with knee joint involvement was operated and the outcome was satisfactory. Four other patients were shifted on second line drugs (after drug susceptibility test and proven to be MDR-TB), three of whom responded and one was lost to follow up (data was included in analysis as the patient received immunomodulation as per our

study). The blood parameters were noted after one month of completion of immunomodulation (6th month of therapy) which showed statistically significant increase in CD4 & CD8 counts ($p=0.04$). Mean CD4 count was $432/\text{mm}^3$ which increased to $747/\text{mm}^3$ while CD8 count showed increment from $458/\text{mm}^3$ to $617/\text{mm}^3$. Mean haemoglobin values, ESR and total leucocyte count showed favourable statistically significant outcome. The increase in total protein in both group A & B was not significant, indicating no role of nutrition in the cell counts variations. Table 2 shows data of non responders (immunomodulated) group

Our study showed, that patients who were nonresponsive to anti tubercular regimen, when given immunomodulation, showed a significant rise in CD4 & CD8 counts ($p=0.04$) and haemoglobin values ($p=0.026$) when compared to pre immunomodulation values. ESR and TLC however were found to decrease significantly ($p<0.05$). Fig. 1 shows radiograph of a 32 years old patient who presented with foot pain and swelling with lytic lesion in medial cuneiform (Fig. 1) patient was started on ATT and didn't improved after 3 months of therapy. Patient was put on immunomodulation. Fig. 2 shows reossification and mineralisation of lesion after immunomodulation. Another patient presented with right sided psoas abscess (Fig. 3) with intact neurology. During the initial course of treatment abscess was aspirated 3 times. Patient was immunomodulated after third aspiration and abscess resolved (Fig. 4). A 28-year-old female presented to OPD with discharging sinus from lateral aspect of distal arm (Figs. 5 and 6) patient was not responding on ATT and was immunomodulated which resulted in healing of sinus and radiological healing of lesion with reossification (Figs. 7 and 8)

4. Discussion

Worldwide, 9.6 million people are estimated to have fallen ill with TB in 2014; 5.4 million men, 3.2 million women and 1.0 million children.² It is estimated that India alone has one fifth of the total world population of tuberculous patients. Thus there are nearly 6 million radiologically proven cases of tuberculosis in India

and perhaps a quarter of these are sputum positive. Of all the patients suffering from tuberculosis nearly 1–3% have the involvement of skeletal system. According to 9th report of WHO Expert committee on tuberculosis, the prevalence of pulmonary tuberculosis is twice as high as the notified cases of 200–350 cases per one lac population. Ideally diagnosis of tuberculosis should be confirmed by demonstration of tubercular bacilli in the skeletal lesion. This is not possible in all cases probably because skeletal tuberculosis is considered to be a paucibacillary disease. In our present series the diagnosis was based on radiologically visible lesion, haematological investigations, histopathological examination and history.

Presence of skeletal involvement in a patient indicates already compromised immune response and hence a need to address this aspect of disease and develop treatment focussing on immunological aspect of tuberculosis. Mycobacterial infection induces changes to weaken the immune-regulatory system in human.

In the present study of 103 cases of osteoarticular tuberculosis majority of the cases 43 (41.7%) were those of spine which compare favourably with other series in Indian patients. Previously the patients with tubercular involvement of various systems were conventionally treated on the three drugs regimen i.e. streptomycin, Isoniazid and PAS/Ethambutol. The duration of treatment varied from 1.5 years to 2 years. Many patients become irregular in taking their prescribed medicine and over half the patients discontinued the treatment much before the prescribed time of 18–24 months. Non-compliance of the patients is primarily based on socio-economic condition and is directly related to duration of treatment. With the advent of Rifampicin and to some extent pyrazinamide with their sterilizing activity has provided the necessary stimulus for evolving short course regimens and need for further modalities to reduce financial and economic burden of disease is imminent.

The concept of intermittent short course therapy originated from a study undertaken at the Madras research Centre (Tuberculosis research Centre, Madras, 1970).¹⁰ The results obtained in Madras prompted the idea of combining short term

Fig. 6. AP and Lateral view of same patient showing involvement of distal humerus.

Fig. 7. Healing of sinus after immunomodulation.

intermittent treatment for tuberculosis. The efficacy of short term chemotherapy is dependent on the rapid and reliable elimination of all sensitive bacilli. Levamisole, ananthelminthic agent has been used in Hodgkin's disease, rheumatoid arthritis, and most recently in adjuvant chemotherapy of colorectal cancer. Levamisole may act by augmenting type 1 response by selective induction of gene transcription of the interleukin-18 E.C. Huskisson¹¹ used levamisole as an immunomodulator in rheumatoid arthritis and found it comparable to d-penicillamine reduce symptoms of disease. Levamisole is thought of being capable of exerting an influence upon various fundamental aspects of the cell mediated immune response, including endocytoses. K pryer¹² in a study on BCG as immunotherapy in bladder cancer found that after repeated instillations for superficial bladder cancer, mononuclear infiltrates were induced in the bladder wall of the patient. Infiltrating cells included T lymphocytes and smaller numbers of macrophages and B lymphocytes. Analysis of the mucosal bladder subpopulations showed that most cells were lymphocytes which were associated with macrophages and eosinophils. The major leucocyte subpopulation was CD4T lymphocytes. Study showed a marked increase in the number of leucocytes, mostly granulocytes, in the urine 24 h

after repeated BCG instillations. Monocytes/macrophages and lymphocytes, mostly CD4T cells were also present. After treatment, the absolute numbers of all subpopulations increased, but the increase in monocytes/Macrophages was most marked, they reached to a conclusion that BCG is superior to IFN α , interferon gamma & IL-2 in enhancing cell-mediated immunity. BCG has been adapted as the therapy for urinary bladder cancer.¹³

A.M. Jackson¹⁴ reported increased levels of cytokines with repeated instillations of BCG in patients with urinary bladder carcinoma, IL-1 β , IL-6, IL-8, IL-10 were increased in urine. As the widely used clinical immunotherapy, BCG eradicates residual tumour in 50% of patients with papillary disease and greater than 70% of patients with CIS

Vijaya Lakshmi¹⁵ conducted the study on children comparing the effect of BCG vaccination on cellular immunity of the children. Subjects were divided into three groups first group consisted of normal children vaccinated with BCG during the first year. Second group consisted of normal children without BCG vaccination while the third group comprised children with active tuberculosis (meningitis, military and lymphadenitis form). The levels of specific CD4 & CD8 counts in the vaccinated group 1 were

Fig. 8. AP and Lateral view 1 month after immunomodulation.

significantly higher ($P < 0.05$) than the levels in the other groups, i.e. unvaccinated group 2 and those with tuberculosis. The difference in interleukin 2 (IL-2) levels between groups 1 and 2 was significant ($P < 0.05$). The magnitude of the response, was albeit, higher in the vaccinated group. Majority (70%) of the BCG vaccines had elevated levels of specific CD4/CD8 ratios, as against a minor (20%) proportion of the unvaccinated, suggesting that BCG vaccine specifically stimulates CD4T cells in children.

As per study by Arora,⁸ CD4 cells produce a variety of cytokines considered to be the central mediators in the genesis of specific T-cell immunity, macrophage activation and granuloma formation, which are the three major recognized components of the protective response against infection with tubercle bacilli. While numerous reports on peripheral CD4 cell counts in patients with pulmonary tuberculosis suggest low or normal values, relatively fewer reports on the CD4 cell count in extrapulmonary tuberculosis have consistently documented lowering of the CD4 cells count which substantiated the immunopathogenic postulation that dissemination of tuberculosis beyond the extrapulmonary site is an indication of failure of the host's immune system to contain the infection at a local site. In their study patients from both groups had a low mean CD4 cell count, as compared with the control group. Most of the patients in group I (93.6%) and in group II (70%) had a significant increase in the CD4 cell count after treatment for three months.

Besides the immunomodulators used in our study, potential of different other modalities as immunomodulators have been explored extensively in literature. Vitamin D3 was found to be an important component of host resistance and TB patients with vit D3 deficiency were not able to mount adequate control of M.tb infection.¹⁶ Montoya¹⁷ and Klug-Micu¹⁸ found vit D3 involved in managing of IFN- γ , IL-32 and IL-15 signaling. During In vitro evaluation of macrophages infected with mycobacterium it was observed that vit D3 instillation resulted in upregulation of both anti inflammatory and pro-inflammatory (IL1B, TNF) genes in 72 h period.¹⁹

It has been postulated that trapping of CD4 cells at a local site of involvement is more selective than that of CD8 cells. Thus, the changes in the profile of peripheral lymphocyte subsets may be brought about by the re-trafficking of cells from the local site to the circulatory pool after healing. CD4T cells are important in the prevention of reactivation and both primary and secondary disease, as decreased CD4T cells accelerate TB in HIV patients.²⁰ CD4T cells are also relatively absent in cavitated TB lesions, HIV/TB coinfecting patients are less likely to have cavitory disease²¹ and CD4-deficient mice have less necrosis in *M. avium*-induced lesions²² suggesting a role for CD4T cells in cavitation. CD4T cells may also reduce bacterial numbers by the induction of apoptosis through the Fas (CD95) ligand system.²³

CD8T cells do play an important role, however, by destroying infected cells or killing intracellular bacteria via the secretion of granulysin and perforin, which lyse host cells and attack Mtb directly.²⁴ Recent studies in the nonhuman primate model, show an important role for CD8T cells in that both BCG-vaccinated or previously infected and antibiotic-cured rhesus macaques had markedly reduced protection against challenge with Mtb after CD8T cell depletion.²⁵

Present study shows that in patients non responsive to anti tubercular regimen, when immunomodulation was done, there was significant rise in CD4 & CD8 counts along with clinical response, signifying helper T-cell count can be seen as a predictor of clinical course. In patients who responded well to first line anti tubercular therapy, though there was increase in CD4 & CD8 counts but it was statistically insignificant after 6 months of therapy, though decrease in TLC and ESR was found to be significant ($p = 0.001$).

The observed relationship between the increase in the peripheral T-cell count and a favourable clinical outcome was further strengthened by the concomitant increase in the haemoglobin level as well as by a decrease in the ESR, total leucocyte count. These changes are all considered to be indicators of successful response to treatment in tuberculosis.

In a study by Bose,²⁶ patients with pulmonary tuberculosis refractory to ATT showed that those who had failure of normalization of T-cell homeostasis ran a risk of relapse, even if they became sputum negative after ATT. These observations are further validated in our study since the normalization of the helper t-cell count showed a correlation with clinical improvement on a long-term basis. Further research should be aimed delineate role of immunomodulation in even responding cases to enhance inherent immune mechanism to fight M. tuberculosis.

5. Conclusion

Treatment of tuberculosis has been a great success after introduction of chemotherapy specifically targeting Mycobacterium tuberculosis. In this modern age of flourishing anti biotic resistance, especially in endemic country like ours there is a need to look treatment of osteoarticular tuberculosis from different perspective. Immunomodulation targets specifically on host immunity to strengthen counter attack over M.tuberculosis.

Relationship between the increase in the peripheral T-cell count and a favourable clinical outcome was further associated with concomitant increase in the haemoglobin level as well as by a decrease in the ESR, total leucocyte count. In our study immunomodulation, has shown a good promise to be of adjunctive use in patients on ATT with non-responsiveness and horizons need to be explored regarding immunomodulation as a tool to treat osteoarticular tuberculosis more efficiently and readily along with first line anti tubercular therapy.

Conflict of interest

The authors have none to declare.

References

- Tuli SM. General principles of osteoarticular tuberculosis. *Clin Orthop Relat Res*. 2002;(May (398)):11–19.
- WHO. *Global Tuberculosis Report 2015*. Geneva: World Health Organization; 2015.
- Golden MP, Vikram HR. Extrapulmonary tuberculosis: an overview. *Am Fam Phys*. 2005;72(9):1761–1768.
- Boom WH, Wallis RS, Chervenak KA. Human mycobacterium tuberculosis reactive CD4T cell clones: heterogeneity in antigen recognition, cytokine production, and cytotoxicity for mononuclear phagocytes. *Infect Immun*. 1991;59:2737–2743.
- Grange JM. *Tuberculosis*. 9th ed. Topley and Wilson Microbiology and Microbial Infections, vol. 3 London: Arnold Publishing; 1998.
- Young D, Hussels T, Dougan G. Chronic bacterial infections: living with unwanted guests. *Nat Immunol*. 2002;3–1026.
- Standford JL, Standford CA. Immunotherapy of tuberculosis with mycobacterium vaccae NCTC 1659. *Immunobiology*. 1994;191(555):563.
- Arora A, Nadkarni B, Dev G, et al. The use of immunomodulators as an adjunct to antituberculous chemotherapy in non-responsive patients with osteo-articular tuberculosis. *J Bone Joint Surg Br*. 2006;88(February (2)):264–269.
- Ray Krishna, Gupta SM, Bala Manju, Murlidhar Sumathi, Kumar Joginder. CD4/CD8 lymphocyte counts in healthy, HIV positive individuals & AIDS patients. *Indian J Med Res*. 2006;124(September):319–330.
- Tuberculosis Chemotherapy Centre. A controlled comparison of a twice-weekly and three once-weekly regimens in the initial treatment of pulmonary tuberculosis. *Bull World Health Org*. 1970;43:143–206.
- Huskinson EC, Dieppe PA, Scott Jane. Immunostimulant therapy with levamisole for rheumatoid arthritis. *Lancet*. 1976;307(February (7956)):393–395 [21].
- Pryor KJ, Goddard, Goldstein' D, et al. Bacillus Calmette-Guerin (BCG) enhances monocyte- and lymphocyte mediated bladder tumour cell killing. *Br J Cancer*. 1995;71:801.
- Bailey & Love's Short Practice of Surgery, 26th edition 1338, ISBN 978-0-340-939376.
- Jackson AM, Alexandroff AB, Kelly RW, Skibinska A, Esuvaranathan K, Prescott S, Chisholm GD, James K. Changes in urinary cytokines and soluble intercellular adhesion molecule-1 (ICAM-1) in bladder cancer patients after bacillus Calmette-Guérin (BCG) immunotherapy. *Clin Exp Immunol*. 1995;99(3):369–375.
- Vijaya Lakshmi V, Kumar Sunil, Surekha Rani H, et al. Tuberculin specific T cell responses in BCG vaccinated children. *Indian Paediatr*. 2005;42(January (1)):36–40.
- Rahman S, Rehn A, Rahman J, Andersson J, Svensson M, Brighenti S. Pulmonary tuberculosis patients with a vitamin D deficiency demonstrate low local expression of the antimicrobial peptide LL-37 but enhanced FoxP3 regulatory T cells and IgG-secreting cells. *Clin Immunol*. 2014;156(2):85–97.
- Montoya D, Inkeles MS, Liu PT, et al. IL-32 is a molecular marker of a host defense network in human tuberculosis. *Sci Transl Med*. 2014;6(250):250ra114.
- Klug-Micu GM, Stenger S, Sommer A, et al. CD40 ligand and interferon-gamma induce an antimicrobial response CD40 ligand and interferon-gamma induce an antimicrobial response against Mycobacterium tuberculosis in human monocytes. *Immunology*. 2013;139(1):121–128.
- Coussens AK, Wilkinson RJ, Martineau AR. Phenylbutyrate is bacteriostatic against mycobacterium tuberculosis and regulates the macrophage response to infection, synergistically with 25-hydroxy-vitamin D(3). *PLoS Pathog*. 2015;11(7):e1005007.
- Wolday D, Hailu B, Girma M, et al. Low CD4⁺ T-cell count and high HIV viral load precede the development of tuberculosis disease in a cohort of HIV-positive Ethiopians. *Int J Tuberc Lung Dis*. 2003;7:110–116.
- Brindle RJ, Nunn PP, Githui W, et al. Quantitative bacillary response to treatment in HIV-associated pulmonary tuberculosis. *Am Rev Respir Dis*. 1993;147:958–961.
- Florido M, Cooper AM, Appelberg R. Immunological basis of the development of necrotic lesions following Mycobacterium avium infection. *Immunology*. 2002;106:590–601.
- Oddo M, Renno T, Attinger A, et al. Fas ligand-induced apoptosis of infected human macrophages reduces the viability of intracellular Mycobacterium tuberculosis. *J Immunol*. 1998;160:5448–5454.
- Stegelmann F, Bastian M, Swoboda K, et al. Coordinate expression of CC chemokine ligand 5, granulysin, and perforin in CD8T cells provides a host defense mechanism against Mycobacterium tuberculosis. *J Immunol*. 2005;175:7474–7483.
- Chen CY, Huang D, Wang RC, et al. A critical role for CD8 T cells in a nonhuman primate model of tuberculosis. *PLoS Pathog*. 2009;5:e1000392.
- Bose M, Gupta A, Banavalikar JN, Saha K. Dysregulation of homeostasis of blood T-lymphocyte subpopulations persists in chronic multibacillary pulmonary tuberculosis patients refractory to treatment. *Tuberc Lung Dis*. 1995;76:59–64.