Evaluation of transfer factor protective efficacy against Tuberculosis in Guinea Pigs

Ekram, A.A. AL-Samarrae1 Khalifa A. Khalifa2 Noria, R.S. Mohammed3
1 Vet. Drugs Research Center- Baghdad.
3 Dep. Microbiology- Baghdad Medicine College.

Summary

Transfer factor has been prepared from sensitized guinea pigs against heat killed Mycobacterium bovis antigen and protective efficacy were evaluated in guinea pigs that gave 1 ml of transfer factor equivalent 5x10⁸ cell / ml of sensitized and normal cell donor; then challenged with dose 0.01 mg / animal of virulent Mycobacterium bovis. After 40 days of challenge all animals were sacrificed.

Histologically: - TFs recipient group showed a mono-nuclear cells infiltration’s (Lymphocytes & Macrophages) with a mild emphysema of lungs, and liver showed mononuclear cells , a small necrotic foci in the lymphocytic organs (spleen & lymph nodes); the kidney showed mononuclear cells in glomeruli and urinary tubules ;While the TFn recipient group were an areas of caseated necrotic materials surrounded by macrophages & Lymphocytes, congestion of internal organs (Liver and Spleen) and Sever congestion with hemorrhage of alveoli tissue, sever emphysema & consolidation in lung kidney showed tuberculle foci in glomeruli and around urinary tubules . There were no pathgenomic changes in heart muscles of TFs recipient group while a mild congestion in TFn recipient group.
Introduction

Tuberculosis (TB) is a chronic zoonotic disease that causes major health problems on global scale of human and animals. Human tuberculosis accounts for 8 million cases of clinical disease with 3 million deaths annually (1).

The reports of WHO estimated that in 1990 about 1.7 million people (one third of world population) infected with tubercle bacilli Bovine (2) tuberculosis causes a major economic loss. (3).

The activity of the classical drugs (first & second line drugs) is limit to eradicate or prevent tuberculosis in infected patients, therefore many studies and researches pointed to the role of immunotherapy as another way or controlling tuberculosis (4). A transfer factor is a dialyzable moiety obtained from immune lymphocytes successfully used for treatment of several infections viral (5); bacterial (6); fungal (5); internal parasites (7) and neoplastic diseases (8).

The aim of this study is to use transfer factor as an immunotherapy protection against tuberculosis in guinea pigs.

Materials and Methods

Transfer factor preparation:-

Ten guinea pigs were immunized by heat killed Mycobacterium bovis antigens (2x 10^8 cell/ ml) mixed with Freund’s incomplete adjuvant (v/v); as a source of transfer factor which was prepared according to (9):-
Spleen cells suspension was centrifuged at 200Xg for 10 minutes and resuspended in PBs. pH7.2; then checked for viability by 0.2 % trypan blue dye, at least 90 of cells were viable. The spleen cell numbers was counted by hemocytometer. (10); the number of cells was adjusted to 5x108 cell/ml. Suspension was freezing and thawing by liquid nitrogen and water bath at 37°C respectively for 10 times. Then centrifugation at 40000 g for 30 minutes supernatant was filtrated through a micron filter (molecular weight < 10,000 dalton), the supernatant equivalent to 5x 10^8 cell / ml, referring to as (TFs) for sensitized (immunized) guinea pigs and (TFn) for normal animals (control group) were prepared as above in the same procedure.

**The protective efficacy of transfer factor in guinea pigs**

Eight guinea pigs were divided into two groups (4 animals in each) as follows: -
TFs recipient group which received intra peritoneally 1 ml of transfer factor equivalent 5x 10^8 cell / ml of sensitized animals.
TFn recipient group which received intra peritoneally 1 ml of transfer factor equivalent 5 x 10^8 cell / ml of normal cell donor.

After 3-5 days of receiving transfer factor, all animals of two groups were challenged by 0.01 mg per animal of virulent Mycobacterium bovis. (11), and sacrificed after 40 days after challenge dose,

Histological changes were investigated of internal organs (lungs, liver, spleen, kidneys, lymph nodes & heart) (12).

**Results**

**Clinical sings :-**

The animals after 40 days of challenge dose by a virulent Mycobacterium bovis, the results showed that the TFs recipient group look like normal in appearance, While the TFn recipient group suffered from weakness, weight & appetite losses, with a difficult respiration.

**Pathological changes: -**

The gross lesions of TFs recipient group are characterized by a slight congestion of liver, spleen, kidney & lung and some animals showed mild emphysema with area of consolidation in the lungs. There was a large bronchial & mesenteric lymph nodes; while in TFn recipient group, there were presence of tubercles of different sizes (diameters ranged from 1-5 mm) in beaded or bulging from surface of internal organs especially liver, spleen & lung.

The lungs appeared different stages of pneumonia with hemorrhagic spots & necrotic foci on the surface with emphysema & consolidation of the epical lobes with congestion of the heart muscle. The liver & spleen appeared sever congestion while the kidney, intestine were congested with an enlargement of mesenteric lymph nodes that appeared white yellowish in color.
Histologically of the internal organs of TFs recipient group showed in the lungs a slight infiltration of mononuclear cells (lymphocytes & macrophages) with a mild congestion & emphysema (Fig. 1)

![Image](image1.png)

**Figure (1) Lung section shows cellular infiltration with congestion and emphysema (H&E x 100)**

The liver showed a slightly infiltration of mononuclear cells with a mild congestion of blood vessels .(Fig.-2).

![Image](image2.png)

**Figure (2) Liver section shows slight cellular infiltration and congestion (H&E x200).**

While the spleen & lymph nodes showed increased mononuclear cells infiltration with a small necrotic foci surrounded by neutrophils ,lymphocytes & macrophages with a mild congestion of blood vessels. (Fig-3).
Figure (3) Spleen section shows slight caseated necrotic material with severe cellular accumulation (H&Ex100)

Kidney showed a slightly infiltration of mononuclear cells in the glomeruli & around urinary tubules (Fig-4).

Figure(4) Kidney section shows cellular infiltration of urinary tubules (H&Ex 200)

In the TFn recipient group the lung showed areas of caseated necrotic materials contained large number of neutrophils, epithelioid cells, fibroblast, lymphocytes & plasma cells with a numbers of giant cells & severe congestion, hemorrhage, emphysema, consolidation of alveolei (Fig-5).
Figure (5) Lung section shows tubercle surrounded by cellular infiltration with emphysema consolidation (H&Ex100)

The liver showed areas of caseated material surrounded by infiltration of mononuclear cells, neutrophils, fibroblasts, and giant cells with dilatation of sinusoids (Fig-6).

Figure (6) Liver section shows tubercle with cellular infiltration and congestion (H&Ex200)

In the Spleen & Lymph Nodes, there was an increase in the septa with cellular infiltration (neutrophils, macrophages) surrounding the large caseated foci with epithelioid cells, giant cells & severe congestion of blood vessels (Fig-7).
Figure (7) Spleen section shows tubercle with severe cellular accumulation(H&Ex100)

The kidney showed different sizes of tubercle foci & dilatation of some urinary tubules containing a proteinous material with mononuclear cell infiltration in glomeruli & around the urinary tubules with congestion of blood vessels. The heart muscle of all animal groups showed no pathogenomic changes (grossly & histologically) except a very mild congestion of some animals of TFn recipient group.

Discussion

The protective immunity of tuberculosis appeared to be a complicated balance between several immunological functions, it is well established that tuberculosis in both human & animals induced a strong cellular response (13).

Transfer factor are a family of highly polar, hydrophilic molecules of low molecular weight approximately 5000 dalton which are produced in small quantities by lymphoid cells & have potent biological activity (9). It is molecules that “educate” recipients to express cell mediated immunity (14).

These molecules are likely to interact with the variable regions of alpha &/or beta chain of T-cell receptors to change their acidity & affinity for antigen in a way that otherwise would only occur after an encounter with antigen (15).

Transfer factor on T-lymphocytes that secondarily modulate monocytes, macrophage response increases interferon activity (16). Transfer factor augments lymphoblast proliferation (response to specific antigen), antigenic specific CD+4 lymphocytes that secrete IL-2. & Other lymphokines which are responsible for positive delayed type cutaneous hyper sensitivity (6) and the role of cytotoxic lymphocytes as one of the mechanisms can be triggered by specific transfer factor (5).
It is found that treatment with transfer factor, an active cell mediated immunity for 17 months can be detected in vivo & in vitro (17).

In our study we use guinea pigs because it is a suitable model for experimental studies of tuberculosis and transfer factor that agreed with (18) referring that the ability of transfer factor prepared from spleen of sensitized and non sensitized guinea pigs to induce delayed type hyper sensitivity in recipient non sensitized guinea pigs protects them against experimental challenge with virulent organism & indicates the role of transfer factor to increase cellular immunity in infection.

The source of transfer factor is very important in the evaluation of immunological response, the dailyssable leukocytes extract (DLE) provokes an increase in the possibility of complete professional response twenty times in the patients that have cell mediated immunity to specific antigen from a positive cell mediated immunity donor. (19).

The pathological changes that are observed in the TFs recipient group showed mild or some times there were no gross pathological changes. There was a lymphocytic stimulation on the other hand & also characterized by absence of gross tuberculous lesions in the internal organs (spleen, liver, lungs, heart, kidney & lymph nodes). In stead of the TFn recipient group that had a miliary tubercle lesions in these organs especially the peritoneal & mesenteric lymph nodes.

Histologically the result shows that was a typical infection of tuberculosis in TFn recipient group which agreed with (20),(21)& (22). While the TFs recipient group had a mild lesions in these organs due to the function of stimulation of specific transfer factor that agreed with (5) &(6).

Finally our conclusion in this study a single dose is not enough to protect against challenge dose of Mycobacterium bovis that agreed with (5).

Reference


