

IMMUNOPATHOLOGICAL EFFECT OF SENSITIZED TRANSFER FACTOR ON THE ORGANS OF GUINEA PIGS AGAINST THEIR CHALLENGE INFECTION WITH *Mycobacterium bovis*

Khalil H.Al-Joboury

Department of pathology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq

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ABSTRACT

In an experimental study was designed to evaluate the immunopathological effect of sensitized *Mycobacterium bovis* transfer factor in guinea pigs organs against challenge infection with these microorganisms.

The results of this study were showed the followings:

1: Transfer factor recipient group: It was showed an early aggregations of macrophages and lymphocytes (early granuloma) in lungs and liver and reactive lymphoid hyperplasia and macrophages proliferation in the paracortical region of mediastinal lymph node and in periarteriolar sheath areas in the white pulps of the spleen (T cell regions). These early granulomas were persisted during 2nd and 4th week postinoculation and slightly decreased and disappeared during the 6th and 8th weeks postinoculation respectively.

2: Group of infection with *Mycobacterium bovis*: It was showed on extensive tuberculous granulomatous lesions in the lungs, liver, spleen, kidneys and in the mediastinal and hepatic lymph nodes. The lesions were initiated at 2nd week postinoculation and it gradually developed into extensive tuberculous granuloma with central caseation, during the 4th and 6th weeks postinoculation. These lesions were persisted and continued during 8th week postinoculation. Two animals died at 7th week postinoculation due to generalized tuberculosis.

3: Transfer factor recipient group and challenged with *Mycobacterium bovis*. It was showed a well developed granulomatous reactions in the lungs, liver, spleen and mediastinal and hepatic lymph nodes. These granulomas consisted of aggregation of epithelioid cells, lymphocytes and few giant cells without caseation. These granulomas were initiated during the 2nd week and gradually increased in size in the 4th week and decreased at 6th week and completely disappeared during the 8th week postinoculation. No animals were died in this group.

4: Control group: It was showed neither morphological and nor histological lesions in the body organs.

Conclusion: These findings indicated that the transfer factor has a protective role in protection of animals against the bacterial challenge .

INTRODUCTION

Transfer factor (TF) is one of lymphokines produced by T lymphocytes following their stimulation by specific antigen (1). It is responsible for transferring delayed type hypersensitivity response from sensitized to non-sensitized individuals (2).

It also act across species in transferring delayed type hypersensitivity.(3)

The most important immunological effect is antigen specific conversion of delayed type hypersensitivity and production of lymphokines.(4)

Transfer factor has been evaluated as a possible therapeutic agent in a number of immunodeficiency disorders, that are associated with viruses, fungi, mycobacterium, leprosy and parasitic infections (5), (6).

For this reason the preset study is aimed to demonstrate the immunopathological effect of the sensitized transfer factor on some body organs following their challenge infection with the Mycobacterium bovis.

MATERIALS AND METHODS

Thirty Guinea pigs, 350-380gms of weight, 3.5 months old, provided by Al-Kindi company for veterinary drugs and vaccine production.

The animals were reared together for two weeks to check them for complete health, all the animals were tuberculin negative. The animals were divided into 4 groups (8 animals per group, except that 4 and 10 animals per the forth group and the second group respectively).

First group; transfer factor recipient group:

These group of animals were intraperitoneally injected with 1ml of sensitized transfer factor against Mycobacterium bovis prepared according to peterson, etal. Method (4).

Briefly two Guinea pigs infected with Mycobacterium bovis, were euthenized and all the spleen cells were harvested. A single cell suspension was made by mascerating spleens on sterile stainless sieve. The cells were adjust to 2×10^8 cell /ml of RPMI-1640 containing 10% fetal calf serum and lysed by freezing and thawing until lysis was complete.

The cells suspensions was centrifuged at 40,000xg for 30 minutes and supernatant was collected, filtered through amicon filter with a 10 μ m membrane. The filtrates were collected and lyophilized until using.

Second group; Mycobacterium bovis infection group:

These groups of animals were infected with Mycobacterium bovis, according to the Niaz and Siddiqi method (7) with some modifications.

Briefly, pure local strain of Mycobacterium bovis was cultured in ungar broth medium for 15 days, and were harvested by centrifugation at 3000 RPM for 30 minutes, washed 3 times with phosphate buffer saline.

Then the bacterial suspension was made (1mg of bacterial precipitate per 1ml of phosphate buffer saline) and injected intraperitoneally into each animals of these group.

Third group; transfer factor recipient and Mycobacterium bovis challenged group: These groups of animals intraperitoneally received, similar dose of transfer factor, used for first group and after 72hours intraperitoneally challenged with similar dose of mycobacterium bovis used for second group.

Fourth group; control group:

This group was intraperitoneally injected with 1ml of phosphate buffer saline.

All the groups of animals were killed at 2nd, 4th, 6th and 8th weeks postinoculation, and all morphological lesions were recorded.

For histopathology, the representative pieces of infected organs were fixed in 10% neutral buffered formalin, processed routinely; tissues were embedded in paraffin. Sectioned at 5 μ m and stained with hematoxylin and eosin.

RESULTS

This study was revealed the followings findings:

First group; transfer factor recipient group:

During the second week postinoculation white foci of inflammation were distributed on lungs and liver. Also hyperplasia of periarteriolar sheath region of the white pulp of spleen and on paracortical regions of mediastinal lymph node (T cell regions for these organs). These white foci of inflammation were consisted of an aggregation of macrophages and few lymphocytes.

The foci were slightly increased in size and became more prominent at 4th week postinoculation, they were consisted of aggregation of macrophages and the number of lymphocytes were increased (fig-1). During the 6th and 8th weeks postinoculation, these foci of inflammation were gradually decreased and completely disappeared respectively.

Second group; Mycobacterium bovis infection group :

During second week postinoculation a white foci of necrosis were distributed on the lungs (fig-2), liver, kidneys and in the cortical regions of mediastinal and hepatic lymph nodes and in the white pulp of spleen (fig-3).

These foci of necrosis were consisted of aggregates of macrophages, neutrophils and edema. These foci of necrosis were gradually increased in size and became more prominent during the 4th and 6th weeks postinoculation forming tuberculous granulomas consisted of a central area of caseation, surrounded by wide zone of epithelioid cells and some langhans giant cells and on the periphery of these inflammatory cellular layer, there was extensive lymphocytes infiltration together with some fibroblasts proliferation which was mostly prominent during the 8th week postinoculation forming an encapsulation of these granulomatous lesions and continued. Two animals of these groups had died at the 7th week postinoculation due to generalized tuberculosis.

Third group; Transfer factor recipient and Mycobacterium bovis challenged group:

This group of animals, during the 2nd week postinoculation was showed a well developed granulomatous reaction, mostly evident on the lungs (fig-4), liver (fig-5), spleen (fig-6) and in the mediastinal and hepatic lymph nodes. These granulomatous reactions were consisted of aggregates of epithelioid cells, some langhan's giant cells and lymphocytes without caseation these well developed granulomas were persisted at 4th week postinoculation and gradually decreased and disappeared during 6th and 8th weeks postinculation.

Fourth group; control group:

This group of animals was showed neither morphological and nor histological lesions in the body organs.

DISCUSSION

The therapeutic effect of transfer factor in infectious diseases and cancers have not been completely identified and the immunological mechanisms of the transfer factor, underlying the antigen specific cell-mediated immune effects (2) which is correlate with the results of the present study, that the transfer factor was induced immunological effects through the activated macrophages (epithelioid cells) and lymphocytes infiltrations in the liver, lungs, spleen and lymph nodes.

The infiltration of macrophages had important role in processing of antigen whereas lymphocytes responsible for lymphokines production. This infiltration is an indication of cell mediated immune response (8,9) which was evident through the granulomatons reactions in the present study.

The lymphokines that were produced by lymphocytes act as an activation factor for phagocytosis and as inhibitors for macrophages movement outside the inflammatory site (site of antigen or Mycobacterium bovis location) in the present study (9).

The role of transfer factor was mostly prominent in group of animals that received transfer factor and challenged with Mycobacterium bovis, these groups of animals showed well developed granulomas and these granulomatous response was explained as an emergence of cell mediated immunity against this microbial infection (10).

These granulomas were consisted of epithelioid cells and lymphocytes which was mostly evident in these group of animals, these granulomatous response is under the effect of transfer factor which had a role in limitation of bacterial proliferation and colonization in the organs, in addition to that transfer factor had a role in activation of macrophages in the form of epithelioid and giant cells (11) and through lymphokines production by the lymphocytes which were extensively infiltrated in these groups of animals.

Also these groups of animals showed no caseation in these granulomatous reactions in lung, liver, spleen and lymph node, the absence of caseation indicate limitation of both bacterial proliferation and colonization in these organs, and therefore, no tissue destruction and no caseation. A similar findings were observed in mice inoculated by transfer factor and challenged with typhoid bacilli (12).

The second group of animals which received infective dose of Mycobacterium bovis, showed a well developed tuberculous lesions in the form of white foci in the lungs, liver, spleen, kidneys and lymph nodes; these lesions primarily were consisted of macrophages, neutrophils and few lymphocytes and edema; these inflammatory lesions were gradually increased to form wide tuberculous granulomatous reaction which persisted for 6th and 8th weeks postinoculation and continued. These tuberculous lesions were also observed by many workers (13), they reported that following inoculation of tubercle bacilli, these bacteria were introduced inside the neutrophils and macrophages for multiplication and destruct them then reintroduce inside the other macrophages and these cells carry them through the lymphatic or blood circulation to other sites in the body organs (11). To produce tuberculous granulomatous reactions which was mostly evident in this group of animals. Also, these granulomatous lesions were contained extensive caseation, these granulomas were persisted and became generalized and caused death of two animals in these groups.

The caseation was resulted from the continuous multiplication of tubercle bacilli in macrophages; causing destruction of tissue at the site of bacterial proliferation, also destruction may be under the effect of hydrolytic enzymes released by macrophages and neutrophils (14) and cytotoxic effect of lymphokines released from activated T cells (15).

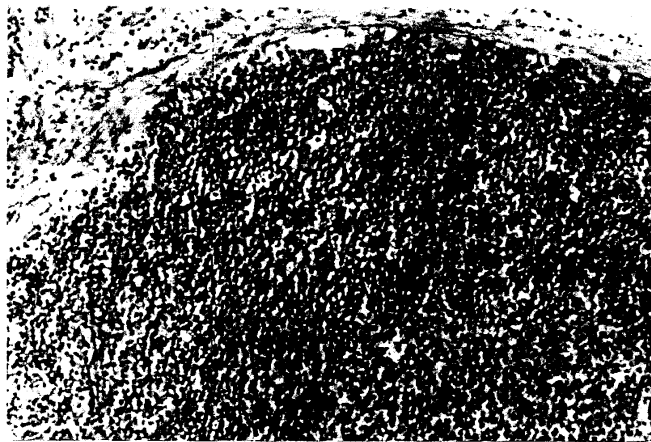


Fig-1: Mediastinal lymph node tissue. There was extensive lymphoid reactive hyperplasia and macrophages proliferation in the paracortical area (T cell region), at the 4th week postinoculation. (H4E) x125.

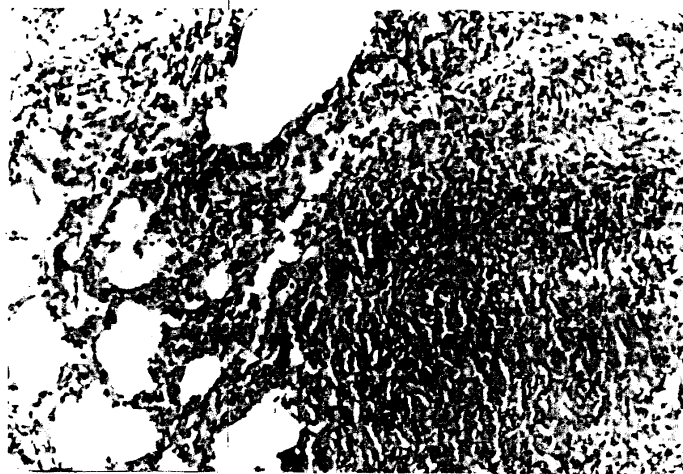


Fig-2: Lung tissue. There was a tuberculous granulomatous lesion with the central caseation (4th week postinoculation). (H4E) 125.

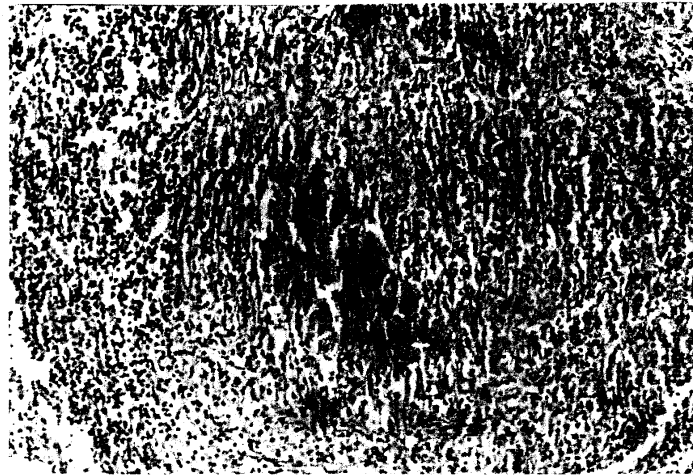


Fig-3: spleen tissue. There was extensive tuberculous granulomatous lesion surrounding the central caseation (6th week postinoculation). (H4E) x125.



Fig-4: Lung tissue. There was a well developed granulomatous reaction without caseation (4th week postinoculation). (Hx E) x125.

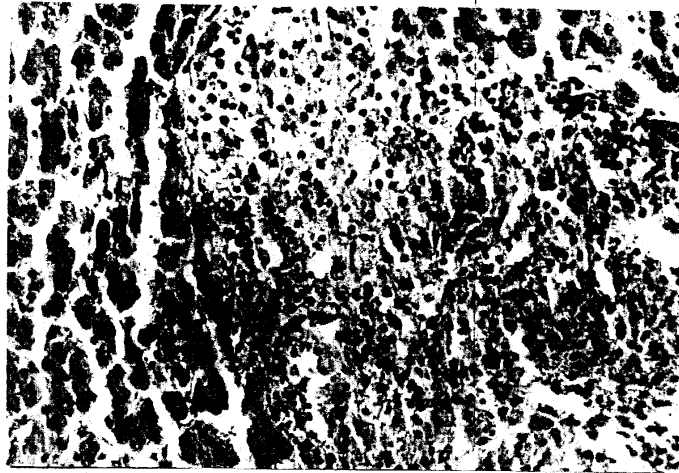


Fig-5: Liver tissue. There was a well developed granulomatous reaction without caseation (4th week postinoculation). (HxE) x125.

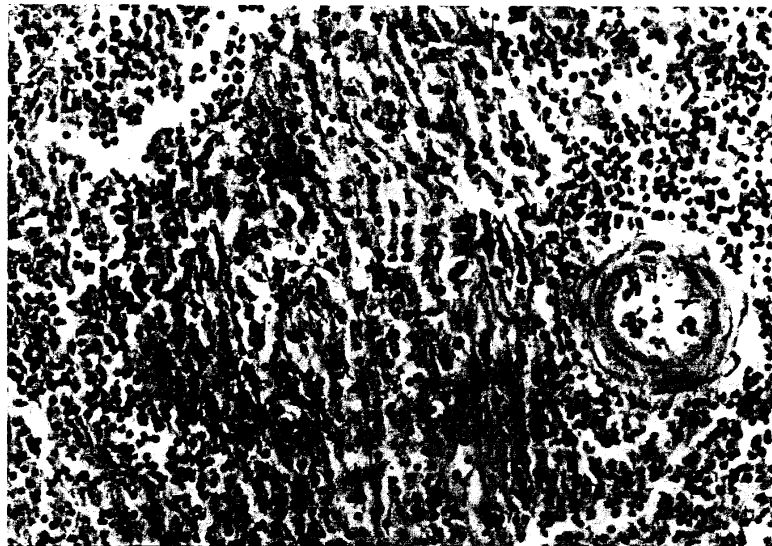


Fig-6: spleen tissue. There was a well developed granulomatous reaction without caseation (6th week postinoculation). (HxE) x250.

دراسة التأثير المرضي للمناعي للعامل الناقل المحسس على أعضاء خنازير غينيا و حمايتها من خمج

التحدي بعصيات السل البقري

خليل حسن زناد الجبوري

فرع الأمراض، كلية الطب البيطري، جامعة بغداد، بغداد، العراق.

الخلاصة

في دراسة صممت لمعرفة التأثير المرضي – المناعي للعامل الناقل المحسس بجراثيم السل البقري على أعضاء خنازير غينيا و ضد خمج التحدي حيث كانت النتائج كالآتي:

المجموعة الأولى: مجموعة استلام العامل الناقل:

حيث بينت وجود أورام حبيبية مبكرة متمثلة بارتشاح البلاعم الكبيرة و الخلايا للمفاوية في الرنتين والكبد مع فرط لمفاوي فعال و تكاثر شديد للبلاعم الكبيرة في مناطق خلايا T في الطحال و العقد للمفاوية المنصفية. إن هذه الأورام الحبيبية المبكرة قد ظهرت خلال الأسبوع الثاني من الحقن و استمرت خلال الأسبوع الرابع و قد صغر حجمها تدريجيا في الأسبوع السادس و اختفت تماما في الأسبوع الثامن من الحقن.

المجموعة الثانية: مجموعة الخمج بجراثيم السل البقري:

حيث بينت وجود أورام حبيبية تدرنية في الرنتين، الكبد، الطحال، الكلى و في العقد للمفاوية المنصفية و الكبدية. إن هذه الأفات التدرنية قد بدأت في الأسبوع الثاني من الحقن و تطورت إلى أورام تدرنية واسعة ذات مراكز نخرية متجينة في الأسبوعين الرابع و السادس من الحقن. إن هذه الأفات التدرنية قد استمرت خلال الأسبوع الثامن من الحقن مؤدية إلى هلاك حيوانات نتيجة لحدوث مرض السل المنتشر في كافة الأعضاء.

المجموعة الثالثة: مجموعة استلام العامل الناقل مع خمج التحدي بجراثيم السل البقري:

حيث بينت وجود أورام حبيبية متطورة جيدا في الرنتين، الكبد، الطحال و العقد للمفاوية المنصفية و الكبدية إن هذه الأورام الحبيبية تكونت أساسا من تجمع الخلايا الظهارانية (البلاعم الكبيرة النشطة) مع بعض الخلايا العملاقة و عدد هائل للخلايا للمفاوية و بدون أن يحدث تخر تجيني في مراكزها. إن هذه الأورام الحبيبية المتطورة قد ظهرت في الأسبوع الثاني من الحقن و ازداد حجمها في الأسبوع الرابع و قلت تدريجيا في الأسبوع السادس و اختفت تماما في الأسبوع الثامن من الحقن و لا توجد حالة هلاك لهذه الحيوانات في هذه المجموعة.

المجموعة الرابعة: مجموعة السيطرة:

لم تسجل أي آفات عيانية أو مجهريّة و لا حالة هلاك في حيوانات هذه المجموعة. بينت النتائج أن للعامل الناقل دور في تقليل و إنهاء خمج التحدي بعصيات السل البقرية.

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