

IMMUNOPATHOLOGICAL EFFECT OF SENSITIZED TRANSFER FACTOR ON THE ORGANS OF WHITE MICE AGAINST *SALMONELLA TYPHI* CHALLENGE INFECTION.

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Summary

In an experimental study to evaluate the immunopathological effect of transfer factor on the reticuloendothelial organs of white mice and their protection against their challenge infection with *Salmonella typhi*. The results of this study were showed the followings: 1. Transfer factor recipient group: it was showed an early granulomatous lesions in the liver. Reactive hyperplasia in the T cell regions of the spleen and mediastinal lymph node. The early granulomas were persisted during 7th day and slightly regressed on 14th day postinoculation. 2. Infected group with *S. typhi*: It was showed a multifocal microabscesses consisted of aggregates of neutrophils in the focal area of necrosis, which was evident during 7th day and gradually transform into granulomas on 14th day postinoculation. 3. Transfer factor recipient and challenge infection group: It was showed well-developed granulomatous reactions, which indicate an emergence of cellular immunity (delayed type hypersensitivity reaction). These granulomas were more evident on 7th day and slightly regressed on 14th day postinoculation; providing a transfer factor role in tissue reaction and termination of infection.

دراسة التأثير المرضي والمناعي للعامل الناقل المحسس بجراثيم التايفونيد على
الأعضاء البطانية - الشوكية في الفئران البيضاء وحمايتها من خمج التحدي.

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الخلاصة

في دراسة صممت لمعرفة التأثير المرضي - المناعي للعامل الناقل المحسس بجراثيم التايفونيد على الأعضاء البطانية - الشوكية في الفئران البيضاء وحمايتها من خمج التحدي، حيث كانت النتائج كالاتي: مجموعة استلام العامل الناقل: حيث بينت وجود أورام حبيبية مبكرة في الكبد، الطحال والعقد اللمفاوية المساريقية وهذه الأورام كانت واضحة في اليوم السابع وتخفت تدريجيا في اليوم الرابع عشر من الحقن. مجموعة خمج التحدي: حيث بينت وجود خراجات مجهريّة متعددة في الكبد والطحال والعقد اللمفاوية المساريقية، تميزت بارتشاح العدلات الكثيف في المنطقة النخرية. حيث كانت

واضحة في اليوم السابع وفي اليوم الرابع عشر من الحقن قد تحولت الى اورام حبيبية ولتستمر. مجموعة استلام العامل الناقل مع خمج التحدي: حيث بينت وجود أورام حبيبية متطورة في الكبد والطحال والعقد اللمفاوية المساريقية، حيث كانت واضحة في اليوم السابع وبدنت تختفي تدريجيا في اليوم الرابع عشر من الحقن مبينة ظهور المناعة الخلوية بوجود العامل الناقل ودورها في حماية الفئران من خمج التحدي.

Introduction

Transfer factors (TF) are proteins that transfer the ability to express antigen specific cell mediated immune response from immune donors to nonimmune recipients⁽¹⁾. To date, it has been demonstrated that they activate the effect or mechanism of the cell mediated immune system and that they have no significant effect on the B cells mediated immune function⁽²⁾ Transfer factors have been used for immunotherapy for a number of immunodeficient disorders that are associated with persistent or recurrent infections with viruses, fungi, mycobacteria and intestinal parasites⁽³⁾. The present experiment was aimed to test the immunopathological effect of sensitized transfer factor (prepared in Guinea pigs against *Salmonella typhi* antigens) in the reaction against the challenge infection in white mice with this microbe.

Materials and Methods

Sixty white albino mice, 20gm of weight and two months old, provided by Al-Kindi Company for Veterinary drugs and vaccines production. The animals were reared together for 2 weeks to ensure for their complete health. Then divided into 4 groups (equally).

1. Transfer factor recipients group:

This group of white mice was intraperitoneally injected with 1ml of transfer factor (single dose; 1 ml equivalent to 5×10^8 spleen cells). Transfer factor prepared from sensitized spleen cells against whole cell killed *Salmonella typhi* vaccine (as a killed antigen) and according to Petersen *et al.* method⁽⁴⁾. Briefly, all the spleen tissues were taken from 4 Guinea pigs immunized against killed whole cell typhoid vaccine. A single cell suspension was made by macerating spleens on sterile stainless sieve. The cells were adjusted 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 cell suspensions were centrifuged at 40,000 g for 30 minutes and supernatant was collected, filtered through amicon filter with 10mm membrane. The filtrates were collected and lyophilized until using.

2. Group of *S. typhi* infection:

This group of animals was intraperitoneally injected with 0.25ml of trypticase soy broth culture of *S. typhi*, washed in phosphate buffer saline, and containing 19^9 bacterial cell /ml and their LD50 dose is corresponding to 2.5×10^7 bacterial cell, identified according to Miles and Misra method⁽⁵⁾.

3. Transfer factor recipient and *S. typhi* challenged group.

This group of animals received the similar dose of transfer factor and after 24 hrs was intraperitoneally injected with the similar dose of *S. typhi* that had given into infected group.

4. phosphate buffer saline recipient group (control group) received 0.25ml of phosphate buffer saline intraperitoneally.

During 7th and 14th days postinoculation, all these groups of animals were sacrificed (using jar containing ether) and the reticuloendothelial organs (liver, spleen and mediastinal lymph node) were taken and examined for morphologic lesions and small pieces of infected tissue were fixed in neutral buffered formalin; processed routinely and cut at 5 M thickness and stained with hematoxylin and eosin.

Results

This study was revealed that there was a prominent effect of transfer factor on tissue reaction in the reticuloendothelial organs ; during the 7th and 14th days postinoculation.

1. Transfer factor recipient group:

There was an infiltration of mononuclear cells (lymphocytes and macrophages); forming an early granulomatous type lesion. This inflammatory reaction was seen in the area adjacent to sinusoids and replacing the hepatic cords in the liver tissue. Also, reactive hyperplasia was seen in periarteriolar sheath area in white pulp of spleen (Fig.1) and in parafollicular area of mediastinal lymph node (Fig.2), (T cell regions for both organs). Both of these cellular reactions were persisted and gradually regressed on 14th day postinoculation.

2-Group of *S. typhi* infection:

This group of animals was showed a multifocal microabscess in the liver tissue (Fig.3), in the white pulp of spleen (Fig.4) and in cortical region of mediastinal lymph node. The microabscess was consisted of focal area of necrosis infiltrated with neutrophils and on the periphery of the necrosis some lymphocytes

and macrophages infiltration. Also there was an extensive congestion of the tissues of these organs. Both of these microabscesses were persisted and gradually transformed into granuloma on 14th day postinoculation.

3-Transfer factor recipient and *S. typhi* challenged group:

This group of animals was showed too much amount of inflammatory reaction in the reticuloendothelial organs. The tissue reaction was consisted of an advance granuloma consisted of an extensive infiltration of lymphocytes and macrophages with mild centrally located area of caseous necrosis which was evident in some tissue sections. Some of these granulomatous lesions were also infiltrated with either epithelioid or giant cells. These granulomatous reactions were seen replacing hepatic cords and in portal regions of liver tissue (Fig.5), and also it was seen replacing the whole white pulp of spleen (Fig.6) and most of cortical region of lymph node. Both of these granulomatous reactions were persisted and gradually regressed on 14th day postinoculation.

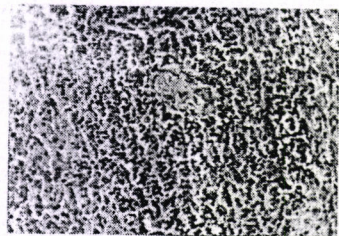


Fig.1: Spleen tissue was showed a reactive hyperplasia in the periarteriolar sheath area in white pulp (T cell region), during 7th day postinoculation. (H&E) X 250



Fig.2: Lymph node tissue was showed a reactive hyperplasia in paracortical area (T cell region), during 7th day postinoculation. (H&E)X125

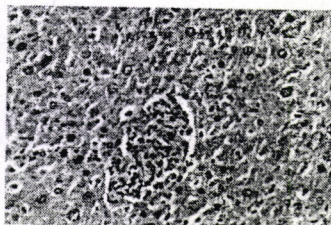


Fig.3: Liver tissue was showed a microabscess consisted of aggregation of neutrophils in the necrotic area, during 7th day postinoculation.(H&E) X250



Fig-4: Spleen tissue was showed a microabscess involving whole area of white pulp, during 7th postinoculation.(H&E) X250

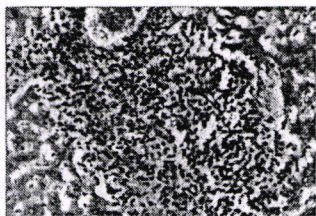


Fig-5: Liver tissue was showed a well developed granuloma replacing hepatic cord, during 14th day postinoculation.(H&E) X250

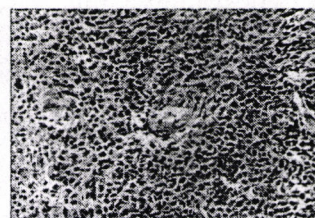


Fig.6: Spleen tissue was showed well developed granuloma replacing whole white pulp, during 14th day postinoculation. (H&E) X250

Discussion

The immunological effect of transfer factor was underlying the antigen-specific cell mediated immune reaction⁽⁴⁾. The results of this study were revealed that the transfer factor had a prominent effect on tissue reaction in the different reticuloendothelial organs. These results were consistent with the previous reports⁽⁶⁾ on the basis of clonality of cytokine producing cells (lymphocytes and macrophages) under the effect of transfer factor. The proliferation and infiltration of these cells in the present study may provide a further evidence for the role of these activated cells in cellular immunity conferred by the transfer factor.

The infected group of animals was showed multifocal microabscesses, were mostly evident in the reticuloendothelial organs. The microabscess consisted of local necrosis, infiltrated by the neutrophils with some of lymphocytes and macrophages. Some features of the histopathological findings were also observed in the organs of mice infected with this type of organisms⁽⁷⁾ and with *S. typhimurium*, another related microorganism^(8,9). Both of those workers explained that these microabscess occurred in relation with these microorganisms on the basis of rapid proliferation of the microorganism at the inoculation sites and early dissemination into reticuloendothelial organs. There was no apparent involvement of macrophages and lymphocytes in the lesions at the early stage of the disease; the similar findings were observed in mice infected with similar type of organism⁽⁷⁾ and with other related organism, *S. typhimurium* and *S. paratyphi-A* and B^(9,10,11). Both of those workers observed extensive lymphocytes and macrophages infiltration, replacing the neutrophils and following the 10th day postinoculation to form granuloma which was not seen early in the infected

group. Also, they reported that the transformation into granuloma was undoubtedly indicative of emergence of delayed type hypersensitivity, which was seen early in transfer factor recipient group. Also, this study revealed that extensive lymphocytes and macrophages infiltration with the mild centrally located caseated areas in the reticuloendothelial organs forming an extensive granuloma which was well developed in transfer factor recipient and *S. typhi* challenged group. The disappearance of microabscess in this group and appearance of well developed granuloma may be relate to the prominent role of transfer factor on emergence of cell mediated immune reaction which was mostly evident against challenge infection by this microorganism and therefor, the transfer factor had a prominent protection role against challenge infection by this microorganism; this protection was accelerated by early granuloma mediated by delayed type hypersensitivity reaction which was undoubtedly beneficial to the host defence under the effect of transfer factor in termination and regression of the lesion later on. The similar findings were reported in mice following immunization with avirulent *S. typhi* vaccine⁽⁸⁾.

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