Protective activity of specific transfer factor against Salmonella typhimurium infection

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Summary

Specific transfer factor (TF) extracted from spleens of sensitized and non-sensitized guinea pigs to study the efficacy of transfer of cellular immunity specific for salmonella . Two groups each of five guinea pigs were used for in vivo TF preparation . The First group was inoculated with 1 ml of aromatic dependent *Salmonella typhimurium* SL 1479 vaccine at a dose of 10^7 cfu/ml intramuscularly twice at two weeks intervals . The second group was injected with trypticase soy broth similarly . These two groups used as a donor for TF_t and TF_n respectively . Twenty one recipient guinea pigs were divided into three groups, the first group was TF_t at a dose of 1 ml equivalent to $5X10^8$ cell intramuscularly three times/2 days intervals , Similarly the second group was given TF_a where as the third group was given PBS- Cell mediated immunity in recipient animal was evaluated by delayed type hypersensitivity – skin test, Macrophage migration inhibition test (MIF) and then challenged with virulent *Salmonella typhimurium*. The TF_t recipient group induced skin test and showed migration indices less than 0.8 and overcome the challenge organism. Contrary to TF_n & PBS recipient groups which did not show any response for skin test and given migration indices more than 0.8 and did not show resistance for virulent *Salmonella typhimurium* .

Introduction

Transfer factor (TF) designates the active principle in viable leucocytes, leucocyte extract, and dialysates of leucocyte extracts with capacity to transfer DTH (1). Smith *et al.,* (2) were the first to report about a successful treatment of the animals

with an antisalmonella transfer factor . Most of the treated mice survived the infection of the pathogenic *S. typhimurium* strain Later Mikula and Pistle (3) managed to isolate and characterize the fractions containing the protective transfer factor effect against *S. typhimurium*. Mikula *et al.,* (4) reported a successful experiment with calves that were treated with antisalmonella transfer factor and infected by the pathogenic *S. typhimurium* strain .

In the present study the role of transfer factor in host defence mechanism against *Salmonella typhimurium* infection in guinea pigs was investigated .

Materials and Methods

Twenty nine guinea pigs (Salmonella free) were used for preparation and evalution of TF. They were reared in separate cages and fed commercial assorted pellets .

A virulent *Salmonella typhimurium* isolated from a calf suffered from acute enteric disease was used for challenge .

Two type of soluble antigens from *Salmonella typhimurium* and *Salmonella dublin* was prepared according to Mitov (5) . These antigens used for delayed type hypersensitivy (DTH) – skin test and macophage inhibition test (MIF) . Protein content was determined using the method of Lowery *et al* (6).

Two groups of G. pigs were used for immunization (5 in each group) , the first group injected with 1 ml of aro *S. typhimurium* SL 1479 vaccine at a dose of 10^7 c.f.u./ml intramuscularly twice at two weeks intervals, this group acted as a donor for TF_t and the scond group (control group) similarly injected with 1 ml of trepticase soy broth (TSB) , the group acted as a donor for TF_n (All. G. pigs were sacrificed three weeks later after the detecting of positive DTH – skin test (14) .

Preparation of transfer factor was performed as described by Rozzo and Kirkpatrick (7) . Toxicity test was determined according to British pharmacopoeia (1993) .

Transfer factor activity assay :-

Nineteen adult healthy G. pigs (Salmonella free) were divided into :

1-TF_t recipients group : Nine animals received TF prepared from immunized animals with aro *S. typhimurium* SL 1479 vaccine which were injected intramuscularly three times at two days intervals in a dose of 1 ml equivalent to $5x10^6$ cell/ml.

2-TF_n recipients group:- Five animals received TF prepared from non immunized animals (normal) which were injected similarly with 1 ml equivalent to $5X10^6$ normal cell/ml .

3-PBS recipient (negative control) group :- Five animals inected similarly sterile 1 ml PBS

The cell mediated immunty in TF recipient groups were checked by :-

A- DTH – skin test : This test was done 24 hours after administration of TF , Skin reaction was recorded 24, 48 and 72 hours after intradermal (i.d) inoculation with soluble antigens.

B- Macrophage migration inhibition test (MIF test)

This test was done on three groups of G. pigs, which received TF_t TF_n and the control , at the 2^{nd} week after the administration of the third dose in according with Weir's method (8) .

Table (1) DTH Skin reaction in TF_t treated G. pigs

Soluble Ag. 100 µg/ml	Diameter of reaction (mm)	TF_t group			TF_n group	PBS group
		24h	48h	72h	24-72 h	24-72h
S.typhimurium	Rang Mean ± SD	11-13 12.2 ± 0.966	8-10 9.2 ± 0.639	4-6 5 ± 0.881	0	0
S.dublin	Rang Mean ± SD	7-9 7.75 ± 0.743	4-6 5.1 ± 0.90	0	0	0
PBS	0	0	0	0	0	0

Challenge :

The control and TF recipient groups, were challenged with 100 LD_{50} of virulent *S. typhimurium* orally five days post TF administration .

The clinical condition was evaluated twice daily, and the dead G. pigs were tested bacteriologically .

Results

1. DTH – Skin reaction ;-

Twenty four hours after administration of the third dose of TF_t ,TF_n, PBS, skin reactivity showed postive results in TF_t recipient groups . While the groups that received TF_n and PBS showed negative results (Table 1) .

2. Macrophage migration inhibition test :-

MIF activity was determined in all G. pigs which received TF_t . These animals showed a mean indices of macrophage migration inhibition (0.220±0.060) , (0.234±0.094) and (0.320±0.130) at the different concentrations of *S. typhimurium* Soluble antigen (100,10,1ug/ml) respectively . The migration indices appear less than 0.8 in TF_t recipient animals . In comparison to the control animals that received TF_n and PBS showed no or very limited inhibition of macrophage migration against all concentrations of antigens . The migration indices appear more than 0.8 (Table 2) . Peritoneal cells from the two groups which were incubated in presence of the PHA (10 ug/ml) showed complete inhibition of migration .

3. Challenge

It was found that post injection of TF to recipient G. pigs at a dose of 1 ml was equivalent to $5X10^6$ cell / ml three times, five days a head of injection of 100 LD_{50} of virulent *S. typhimurium* gave a protection percentage of 80% in TF_t, recipient group, In comparison , no protection was achieved following injection of TF_n, PBS .

The clinical signs were very mild in survived animals from recipient TF_t group, while the control group showed an increase in temperature , pulse , respiration with severe diarrhea , anorxia and death occur within 10 days .

Table (2) Macrophage migration indices in treated G.pigs with TF_t

Groups	Animal no.	Ag Concent. µg/ml			PHA
		100	10	1	10 µG/ML
TFt Recipient	1	0.132	0.105	0.239	0
	2	0.235	0.317	0.404	0
	3	0.264	0.225	0.178	0
	4	0.25	0.291	0.452	0
Mean ± SD		0.220 ± 0.060	0.234 ± 0.094	0.318 ± 0.130	
TFn Recipient	1	0.826	0.911	1.045	0
	2	0.790	0.870	0.954	0
	3	0.863	0.959	1.01	0
Mean ± SD		0.826 ± 0.036	0.913 ± 0.044	1.003 ± 0.045	
PBS (Control)	1	0.813	0.849	0.923	0
	2	0.812	0.850	0.970	0
	3	0.933	0.914	1.000	0
Mean ± SD		0.852 ± 0.069	0.871 ± 0.037	0.964 ± 0.038	

Discussion

The high immunological activity of TF was determined by inducing DTH – skin test in the recipient non sensitized G. pigs , which received TF_t , and gave a pronounced reaction post 1/d inoculation with *S.typhimurium* and *S.dublin* antigens. These results were in agreement with those reported by (9,10,15) .

The results indicated that TF_t recipient group showed inhibition of macrophages migration , both groups showed migration index which was less than 0.8 in comparison to control groups received TF_n or PBS which had migration index more than 0.8 and known as unresponsive at the same concentrations . Similar effects were observed by (10,11,12) Transfer factor is able to transfer not only DTH skin reaction but is also responsible for the production or initiation of other reaction of cell mediated immunity by production of Lymphokines , such as macrophage migration inhibitory factor for (13) .

Results obtained in the present study suggest that TF recipient groups from immunized animals induced protection against oral challenge with virulent *S. typhimurium* , however no protection was observed in the control groups. Similar findings described previously (3,4) . They reported that the application of DLE induced a marked inhibition and/or elimination of penetrative abilities of virulent *S.typhimurium* strain into the liver and spleen as well as colonization of digestive tract in white mice .

This study concluded that three intramuscularly applied doses of TF preparation induced prevention against experimental challenge in recipient G. pigs . The protection was associated with a significant change in the value of cell mediated immunity

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كفاءة العامل الناقل الخاص بالسالمونيلا في نقل المناعة الخلوية

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الخلاصة

تم استخلاص العامل الناقل من طحال خنازير غينيا المحسسة وغير المحسسة لتقييم كفاءته في نقل المناعة الخلوية الخاصة بالسالمونيلا . استخدمت مجموعتين من خنازير غينيا ، حقنت المجموعة الأولى باللقاح المضعف وراثياً بجرعة Aromatic dependent *Saltnonella . typhimurium* SL 1479 10^7 خلية حية / ملليتر في العضل مرتين . أما المجموعة الثانية حقنت بـ 1 ملليتر بمرق فول الصويا (PBS) بنفس الطريقة كمجموعة سيطرة واستخدمت هاتين المجموعتين كواهب للعامل الناقل TF_t , TF_n على التعاقب . ثم حقن العامل الناقل ثلاث مرات كل يومين وبجرعة 1 ملليتر مكافئة إلى 5×10^8 خلية / ملليتر في العضل فأحدثت تفاعل موجب لفحص الحساسية الجلدي المتأخر الخاص بالسالمونيلا .

كما تم استخدام واحد وعشرون خنزير غيني كحيوانات مستلمة غير محسسة قسمت إلى ثلاث مجاميع . المجموعة الأولى حقنت بالعامل TF_t ثلاث مرات وبجرعة 1 مل مكافئة إلى 5×10^8 خلية / ملليتر في العضل أحدث تفاعل موجب لفحص الحساسية الجلدي المتأخر الخاص بالسالمونيلا . كما لوحظ تثبيط في هجرة الخلايا البلعمية والذي أعطى منسب هجرة اقل من 0.8، كما قاومت جرعة التحدي بجرثومة السالمونيلا تايفيميورم الضاربة . في حين لم تظهر المجموعتين المستلمة TF_n , PBS أي استجابة لفحص الحساسية الجلدي المتأخر وأعطت منسب هجرة أعلى من 0.8 ولم تظهر أي مقاومة لجرعة التحدي الضاربة .