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 trypsinase 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 5X10^6 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 antosalmonella transfer factor. Most of the treated mice survived the infection of the pathogenic \textit{S. typhimurium} strain. Later Mikula and Pistle (3) managed to isolate and characterize the fractions containing the protective transfer factor effect against \textit{S. typhimurium}. Mikula \textit{et al.}, (4) reported a successful experiment with calves that were treated with antiscalmonella transfer factor and infected by the pathogenic \textit{S. typhimurium} strain.

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

**Materials and Methods**

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

A virulent \textit{Salmonella typhimurium} isolated from a calf suffered from acute enteric disease was used for challenge.

Two type of soluble antigens from \textit{Salmonella typhimurium} and \textit{Salmonella dublin} was prepared according to Mitov (5). These antigens used for delayed type hypersensitivity (DTH) – skin test and macrophage inhibition test (MIF). Protein content was determined using the method of Lowery \textit{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 \textit{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$_1$ and the second group (control group) similarly injected with 1 ml of trepticate 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-TF1 recipients group: Nine animals received TF prepared from immunized animals with ar 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-TFn 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 injected similarly sterile 1 ml PBS

The cell mediated immunity 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 \( \text{TF}_1 \), \( \text{TF}_n \) and the control, at the 2nd week after the administration of the third dose in according with Weir's method (8).
<table>
<thead>
<tr>
<th>Soluble Ag, 100 μg/ml</th>
<th>S. typhimurium</th>
<th>S. dublin</th>
<th>PBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter of reaction (mm)</td>
<td>Rang Mean ± SD</td>
<td>Rang Mean ± SD</td>
<td>0</td>
</tr>
<tr>
<td>TF&lt;sub&gt;n&lt;/sub&gt; group</td>
<td>24 h</td>
<td>48 h</td>
<td>72 h</td>
</tr>
<tr>
<td></td>
<td>11-13</td>
<td>12.2</td>
<td>9.2</td>
</tr>
<tr>
<td></td>
<td>± 0.966</td>
<td>± 0.639</td>
<td>± 0.881</td>
</tr>
<tr>
<td>TF&lt;sub&gt;f&lt;/sub&gt; group</td>
<td>24-72 h</td>
<td>24-72 h</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1: DTH Skin reaction in TF<sub>f</sub> treated G. pigs.
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 positive 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 TFt

<table>
<thead>
<tr>
<th>Groups</th>
<th>Animal no.</th>
<th>Ag Concent. µg/ml</th>
<th>PHA 10 µG/ML</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td>TFtRecipient</td>
<td>1</td>
<td>0.132</td>
<td>0.105</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.235</td>
<td>0.317</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.264</td>
<td>0.225</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.25</td>
<td>0.291</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td>0.220 ± 0.060</td>
<td>0.234 ± 0.094</td>
</tr>
<tr>
<td>TFnRecipient</td>
<td>1</td>
<td>0.826</td>
<td>0.911</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.790</td>
<td>0.870</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.863</td>
<td>0.959</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td>0.826 ± 0.036</td>
<td>0.913 ± 0.044</td>
</tr>
<tr>
<td>PBS (Control)</td>
<td>1</td>
<td>0.813</td>
<td>0.849</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.812</td>
<td>0.850</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.933</td>
<td>0.914</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td>0.852 ± 0.069</td>
<td>0.871 ± 0.037</td>
</tr>
</tbody>
</table>
Discussion

The high immunological activity of TF was determined by inducing DTH – skin test in the recipient non sensitized G. pigs, which received TF₁, 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 TF₁ recipient group showed inhibition of macrophages migration, both groups showed migration index which was less than 0.8 in comparison to control groups received TF₁ 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.
References


11-Khalifa A. K. and Al- Azzawi. W.A. " Extraction of transfer factor and detecting its efficiency in brucella infection " 1 st.


كفاءة العامل الناقل الخاص بالسالمونيلا في نقل المناعة الخلوية

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الخلاصة

تم استخلاص العامل الناقل من طحال خنازير غينيا المحصنة وغير المحصنة
لتقييم كفاءته في نقل المناعة الخلوية الخاصة بالسالمونيلا. استخدمت مجموعتيين من
خنازير غينيا، حققت المجموعة الأولى باللقاح المضمن وراثيًا
بجرعة Aromatic dependent Salmonella Typhimurium SL 1479
10^7 خليية حبة / ملليتر في العضل مرتين. أما المجموعة الثانية حققت بـ 1 ملليتر بمرق
فول الصويا (PBS) بنفس الطريقة كمجموعة سيطرة واستخدمت هاتين المجموعتيين
كواهب للعامل الناقل. ثم حقن العامل الناقل ثلاث مرات كل
يومين وبجرعة 1 ملليتر مكافئة إلى 5x10^6 خليية / ملليتر في العضل فوجدت تفاعل
موجب لفحص الحساسية الجلدية المتأخر الخاص بالسالمونيلا.

كما تم استخدام واحد وعشرون خنازير غيني كحيوانات مستلمة غير محصنة قسمت إلى
ثلاث مجموعات. المجموعة الأولى حققت بالعامل TFn ثلاث مرات وبجرعة 1 مل مكافئة
إلى 5x10^6 خليية / ملليتر في العضل. تفاعل موجب لفحص الحساسية الجلدية
المتأخر الخاص بالسالمونيلا. كما لوحظ تثبيط في هجرة الخلايا البلعمية والذي أعطى
نسبة تهيج أقل من 0.8، كما قام فريق بجرعة التحدي بجرحثة السالمونيلا تافيفيروم
الضارة. في حين لم تظهر المجموعتين المستلمة PBS و TFn
الحساسية الجلدية المتأخر وأعطت نسبة تهيج أعلى من 0.8 ولم تظهر أي مقاومة
لجرعة التحدي الضارة.

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