Bas.J.Vet.Res.Vol. 2No. 5,2006.

ASSESSMENT OF CELLULAR IMMUNITY IN GUINEA PIGS FOLLOWING THE TRANSFER FACTOR AND SENSITIZED SPLEEN CELL TRANSFER USING ERYTHROCYTES: LYMPHOCYTES ROSETTE TEST

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(Received 13 february 2006,Accepted 5 July 2006)

Key words: Immunity,Transfer factor,Rosette test.

ABSETRACT

In an assessment of cellular immunity in Guinea pigs following transfer factor and sensitized spleen cell transfer using the erythrocytes: lyphocytes rosette test (E-rosette test).

The results revealed the following:

There is increase in the mean number of E-rosette forming active and total T lymphocytes in the sensitized transfer factor and sensitized spleen cell recipient groups comparable to control groups received normal spleen cell extract and normal spleen cells. These findings indicate that E-rosette forming cells are of cellular immunological sinificance.

INTRODUCTION

Mammilian lymphocytes have the property of forming loose associations in vitro with hetrogenous erythrocytes. This property in specifically immunized animals can be correlated with the immune condition (1) and in these instances in which the red cell have been sensitized with antibody, the presence of FC receptors on the lymphocytes can be deduced. However, the capacity to form rosette is not limited to these situations and the so-called spontaneous rosette forming cell has also been described in several species of animals (2,3), it has shown that a small proportions (<1%) of spleen cell (T lymphocytes) from mouse will form rosette with sheep erythrocytes, whereas, in man it appears that all or nearly all of the blood T lymphocytes will form rosettes with sheep red blood cells (4). Also, the T cells had rosette forming ability with homologous in addition to heterologous red blood cells. The biological significance of a such high proportion of spontaneous rosette forming cells is not known. For this reason, the present study was aimed to assess the cellular immunity in Guinea pigs following the transfer factor and sensitized spleen cell transfer using Erythrocytes: lymphocytes rosette test (E-rosette Test).

MATERIALS AND METHODS

Four Guinea pigs were immunized by subcutaneous injection of killed whole cell vaccine against Salmonella typhi. Also another four Guinea pigs were injected with phosphate buffer saline as a control. All the spleens were taken for preparation of sensitized cells and transfer factor.

Preparation of Sensitized Spleen cell and transfer factor:

Four Guinea pigs in the immunized group which showed high level of cell mediated immunity (DTH-skin test was >5mm and Migration Index <0.80) were chosen as donors for the sensitized spleen cell and transfer factor.
Four healthy control animals were also chosen as donor for normal spleen cells and normal spleen cell extract. The spleen from both groups of animals was surgically taken out in RPMI-1640 medium and after trimming off all the adherent tissues, cut into small pieces, minced and teased on a sterile stainless sieve to obtain single cell suspension. The leukocytes from the spleen cell suspension were made free from erythrocytes by treatment with 0.83% ammonium chloride, after that spleen cells suspension were washed in RPMI-1640 medium. The spleen cell suspension was checked for viability using 0.2% trypan blue and the number of the spleen cells was adjusted to $5X10^8$ cell/ml RPMI-1640 using neubar chamber (5) and used a viable cells for transferring immunity and for preparation of transfer factor.

Transfer factor preparation:

Transfer factor was prepared according to Petersen etal. Method (6). Briefly, Spleen cell extract of either immunized or control animals were disrupted in phosphate buffer saline. The cell suspensions were centrifuged to remove cellular debris at 2000xg for 10 minutes. The suspensions were frozen in liquid nitrogen and thawed in a 37°C water bath for 10 times. The suspensions were centrifuged at 40.000xg for 30 minutes with a μm 10 membrane (Amicon Inc. Lexington, mass). The filtrates (molecular weight <10.000) were collected and stored by lyophilization until used.
Reconstitution was done in phosphate buffer saline to a concentration equivalent to $5X10^8$ cell/ml and referred as sensitized transfer factor for immunized group and normal transfer factor (normal cell extract) for control group. Both transfer factor preparations were stored at -20°C until using.

Treatment of animals with spleen cells and transfer factor preparations:

1. Sensitized spleen cell recipient (SC recip.) group (4 guinea pigs) intraperitoneally injected with $5X10^8$ cell/ml (one dose).
2. Normal spleen cell recipient (NC recip.) group (4 guinea pigs) intraperitoneally injected with $5X10^8$ normal cell/ml. (one dose).
3. Sensitized transfer factor recipient (TFt) group (4 guinea pigs) intraperitoneally injected with 1ml of TFt equivalent to $5X10^8$ cell/ml.
4. Normal spleen cell extract recipient (TFn) group (4 guinea pigs) intraperitoneally injected with 1ml of normal spleen cell extract.

Erythrocytes: Lymphocytes rosette Test:

This test was done during the 5^{th} to 14^{th} days after the above treatments according to Salbolovic etal technique (7). Briefly:

Lymphocytes preparation:

Lymphocytes were separated from peripheral blood by careful layering on lymphopreb and centrifuging at room temperature for 20 minutes at 400xg. The lymphocytes were removed frome above the lymphopreb surface and washed 3 times in RPMI-1640 Medium and

made up a concentration of $4X10^6$ lymphocytes/ml and the cellular viability was tested with tryban blue stain.

Erythrocytes preparation:

Two ml of heparinized blood from guinea pigs were suspended in 10ml phosphate buffer saline and washed 3 times in phosphate buffer saline and were resuspended in RPMI-1640 medium at a concentration of $40X10^6$ cell/ml.

Rosette preparation:

For rosette preparation 0.25ml of isolated lymphocytes suspension were mixed with 0.25ml of erythrocytes suspension in the round bottom test tube in the presence of 10% fetal calf serum. The tubes were incubated at 37°C for 15 minutes before centrifugation at 200xg for 5 minutes at room temperature the cell pellets were resuspended by gentle rocking and equal amounts of cell pellets suspension were mixed with the 0.2% trypan blue on slide and a total of 200 lymphocytes were counted to find number of rosette forming cells (Active T cell). Other cell pellets suspension were incubated at 4°C overnight and counted the number of total T cells forming rosette.

RESULTS

This study revealed that the rosettes forming cells (active and total T lymphocytes) were increased in all animals received sensitized spleen cells and sensitized transfer factor (Table-1). The mean number of rosettes forming cells (active and total T lymphocytes) was 0.625±0.250 and 0.750±0.288 respectively before treatment of animal. It increased to a mean of 14.250±1.708 and 16.500±1.118 for both rosettes forming active and total T lymphocytes respectively in sensitized transfer factor recipient group, whereas, the mean number of rosettes forming active and total T lymphocytes were increased to 12.250±2.872 and 13.500±3.109 for active and total T lymphocytes respectively in the sensitized spleen cells recipient group. As for, the control groups (normal spleen cell extract and normal spleen cell recipient groups) the mean number of rosettes forming active and total T lymphocytes were 0.625±0.250 and 0.750±0.288 respectively before treatments. It increased to 2.250±0.500 and 2.500±0.577 for active and total T lymphocytes respectively in normal spleen cell extract recipient group and increased to 1.250±500 and 1.500±0.577 respectively in the normal spleen cell recipient.

Table 1: Mean percentage values of active and total T-lymphocytes in the recipient groups (E-rosette Test)

Pretreatment period		**Post treatment**							
		TFt recip.		**TFn recip.**		**SC recip.**		**NC recip.**	
AT	TT	AT	TT	AT	TT	AT	TT	AT	TT
0.625 ± 0.250	0.750 ± 0.288	14.250 ± 1.708	16.5 ± 1.118	2.250 ± 0.500	2.500 ± 0.577	12.250 ± 2.872	13.500 ± 3.109	1.250 ± 0.500	1.500 ± 0.577

*AT = Active T lymphocytes %.
**TT = Total T lymphocytes %.

DISCUSSION

The purpose of this study was to illustrate the capacity of active and total T cells to form rosettes which is widely used and mostly predominant in groups that received sensitized transfer factor (TFt) and sensitized spleen cells (Sc) than control groups received normal cell extract (TFn) and normal spleen cells (Nc). These results indicate an immunological significance for these rosettes formations. Also, under certain circumstances be used to quantitate T and B lymphocytes in a variety of species. Further work on E. rosette forming cells was done and revealed more surface markers for the lymphocytes population, among these cell surface markers is presence of surface immunoglobulin on B lymphocytes and T cell markers (CD_2) on peripheral T lymphocytes and thymocytes (8). Also, more systematic approach to the factors which affect rosette formation would be useful (7). The capacity to form rosette with erythrocytes is restricted mainly to lymphocytes, although, there is certain fact that cultured fibroblasts may under certain circumstances may form rosettes argues against an exclusively imunological significance for the phenomenon and therefor, it is difficult to see an immunological significance in such rosettes (9). Also, the homologous rosette forming capacity by T cells in Guinea pigs in the present study have been described in rabbits (7) to be test equivalent to the E-rosette ability of human T cells (10). It is also possible that the red cells of particular kinds are held at the surface of lymphocytes by the microfilaments (11). Perhaps the lymphocytes-erythrocytes associations in some way related to oxygen carrying potential of the erythrocytes. E-rosettes forming active and total T cells were also increased in some disease conditions (12, 13), with some immunization schedules (14) a similar finding in the present study and even in healthy individuals following their administration the tetramisole as chemical immunopotentiators (15).

CONCLUSION

This study indicates that E-rosette test was of value in detection of cellular immunity in the transfer factor and sensitized spleen cells recipient groups.

تقييم المناعة الخلوية في خنازير غينيا بعد استلامها العامل الناقل و خلايا الطحال المحسسة باستعمال اختبار تكوين الوردة

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الخـلاصـة

في تقييم للمناعة الخلوية في خنازير غينيا بعد استلامها العامل الناقـل و خلايـا الطحـال المحسـة و باستعمال اختبار تكوين الوردة. إذ دلت النتائج إلى زيادة في معدلات أعداد الخلايا التائية الفعالة و الكلية المكونـة للوردة مقارنة بمجموعتي السيطرة المستلمتين مستخلص خلايا الطحال الاعتيادية و خلايا الطحال الاعتيادية. و هذه النتائج تشير إلى أهمية الخلايا التائية في تكوين الوردة كدليل عن وجود المناعة الخلوية.

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