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IDENTIFICATION OF GUINEA PIG T LYMPHOCYTES ON FROZEN TISSUE SECTIONS BY ROSETTE FORMING ASSAY

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Abstract

Guinea pig T lymphocytes were identified on fronzen tissue sections by rosette forming assay, in which neuraminidase-treated rabbit erythrocytes (E_N) were used as the indicator cells. This assay was undertaken by humidity chamber technique. Rabbit E_N adhered to the white pulp of the normal spleen where T lymphocytes occupy preferentially, and in the case of allografted skin rabbit E_N adhered mostly to the epithelial region after 4 and 5 days in guinea pigs. The characteristic adherence of rabbit E_N to guinea pig blood T lymphocytes in suspension was observed by scanning electron microscopy (SEM).

INTRODUCTION

Human T lymphocytes can be identified easily in the peripheral blood by their ability to form rosettes with sheep erythrocytes[1] and neuraminidase-treated erythrocytes (E_N) increase the binding capacity to T lymphocytes[2]. Furthermore, by using the rosette forming assay, identification of T lymphocytes within lymphoid and other tissues has been attempted directly on the frozen tissue sections.[3-10]

On the other hand, it has been reported that guinea pig blood lymphocytes formed specifically rosettes with rabbit erythrocytes and this rosette forming cells were T lymphocytes.[11]

On the bases of these findings, identification of T lymphocytes infiltrating in guinea pig tissues was performed utilizing rabbit E_N on frozen tissue sections in order to elucidate the movement of T lymphocytes which are said to play a central role in cell-mediated immune reactions along with macrophages such as delayed hypersensitivity skin reactions and graft skin rejection.

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MATERIALS AND METHODS

Female English Hartley guinea pigs (B.W. 280–500g) and female Flemish Giant rabbits were used.

(1) Isolation of blood lymphocytes

Guinea pig lymphocytes were isolated from heparinized blood by centrifugation on Ficoll-Isopaque. Cells were washed 3 times in PBS and cell counts were adjusted to 1×10^6/ml in PBS.

(2) Preparation of E_N

Heparinized rabbit blood was collected by cardiac puncture. After removal of the plasma fraction, erythrocytes were washed 3 times in EDTA-gelatin veronal buffer (GVB), 2 times in PBS, 3 times in GVB^{++}, treated with neuraminidase at 37°C for 30 min and resuspended in a final concentration of 1×10^8/ml in fetal calf serum. E_N were kept at 4°C and used within one week after preparation.

(3) Rosette formation of blood T lymphocytes with E_N

One μl of lymphocytes suspension and 10 μl of E_N suspension were added in the pit on the microtest plate (Japan Immunoresearch Laboratory), left at room temperature for 90 min and incubated overnight at 4°C. The specimens, for optical microscopy, were immersed carefully in PBS to wash out nonadherent cells and stained with Giemsa solution. For scanning electron microscopic observation, rosettes were placed on gelatin-coated coverglasses, fixed in 2% glutaraldehyde in PBS for 30 min, washed in PBS and osmicated in 2% osmium tetroxide for 50 min. Then, they were dehydrated in graded ethanols, transferred into isoamyl acetate so as to be critical point dried with Hitachi HCP-1., coated with Au-Pd, and viewed in the SEM (Hitachi HHS-2R).

(4) Preparation of frozen tissues

Normal spleen and allografted skin after 1–12 days in guinea pigs were used. After decapitation, tissue blocks were removed surgically, rinsed in Hank's solution, embedded in n-hexane and quick-frozen in liquid nitrogen. The blocks were maintained at −70°C until sectioning.

(5) Rosette formation on frozen tissue sections

Eight to ten μm thick cryostat tissue sections of frozen tissues were mounted on slides and allowed to air dry. The sections were layered with E_N and incubated in humidity chamber (Lab-Tek Products) at 37°C for 30 min and at 4°C for 5 hrs. The nonadherent erythrocytes were removed by repeated dipping in PBS. Then, for fixation the slides were placed at 4°C overnight in a solution of 0.5% glutaraldehyde containing 1% paraformaldehyde in PBS and washed in PBS. In order to enhance the colour contrast,[9] following procedure was done; first the sections were treated with diamino-

benzidine (DAB) solution added to H_2O_2 for 20 min to stain the indicator cells and washed in PBS, next stained with haematoxylin and eosin. Otherwise, the sections were stained with Giemsa solution.

RESULTS

As shown in Fig. 1, blood T lymphocytes in guinea pig formed rosettes with rabbit E_N. Examining this rosette by SEM, T lymphocyte was anchored to rabbit E_N by long threadlike links (Fig. 2). Rabbit E_N also adhered to the white pulp in guinea pig spleen (Fig. 3), where T lymphocytes occupy preferentially, but scarcely to the red pulp, indicating that guinea pig T lymphocytes formed the rosettes with rabbit E_N on forzen sections. Observing the rosettes formed in allografted skin sections day by day, they were found mostly on days 4 and 5 after grafting and many of them were localized in the epithelial region of the grafts (Fig. 4), although a few were seen in connective tissue.

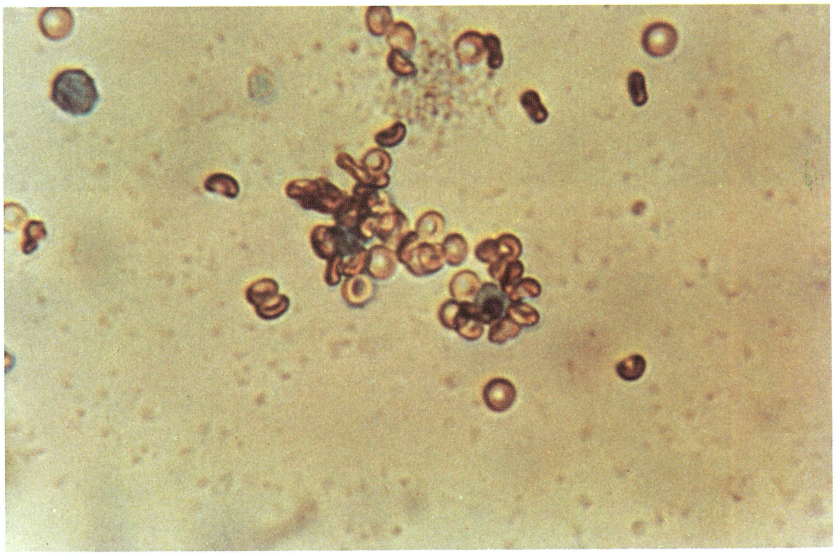

Fig. 1. Rosettes of guinea pig blood T lymphocytes with neuraminidase-treated rabbit erythrocytes. (Giemsa stain ×400)

DISCUSSION

Recently, it is reported that Bjerke *et al.*[9] detected T lymphocytes infiltrating in lichen planus skin lesions by rosette forming assay.

Our results demonstrate that T lymphocytes localized in the tissues of guinea pig can be identified with rosette formation using rabbit E_N in frozen tissue sections, as well as blood T lymphocytes in suspension.

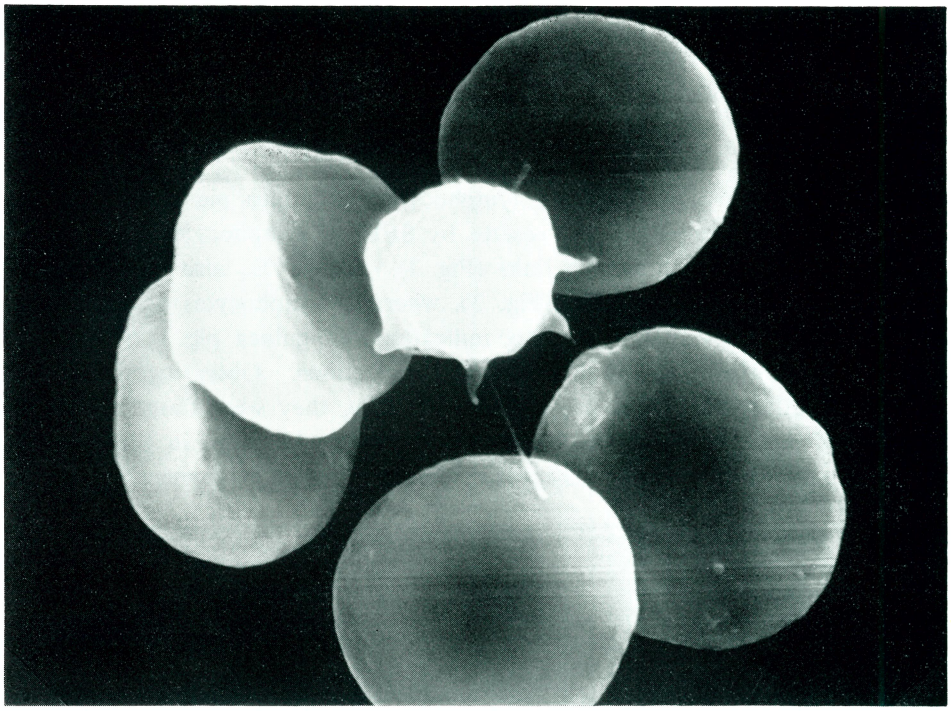

Fig. 2. The view of E_N rosette in suspension by scanning electron microscopy. (×15,600)

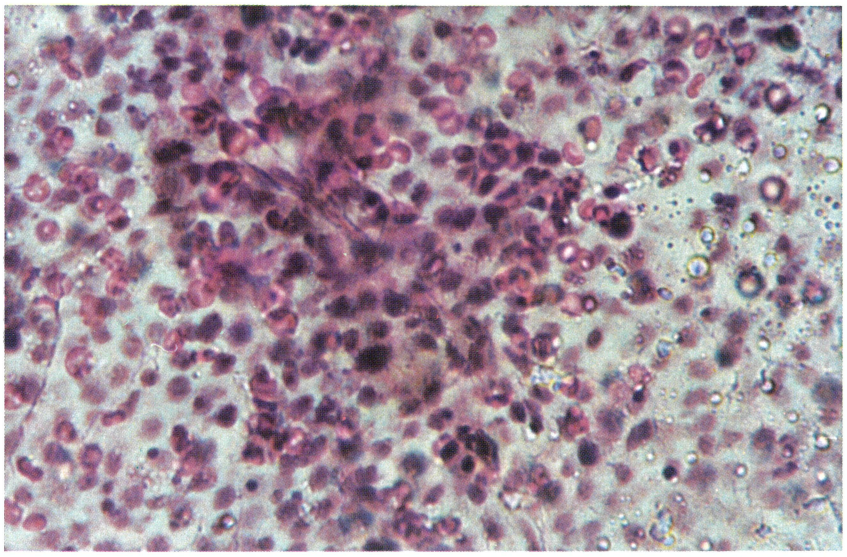

Fig. 3. E_N rosettes in normal spleen. E_N adhered to splenic white pulp in guinea pig. (Benzidine, haematoxylin and eosin stain ×400)

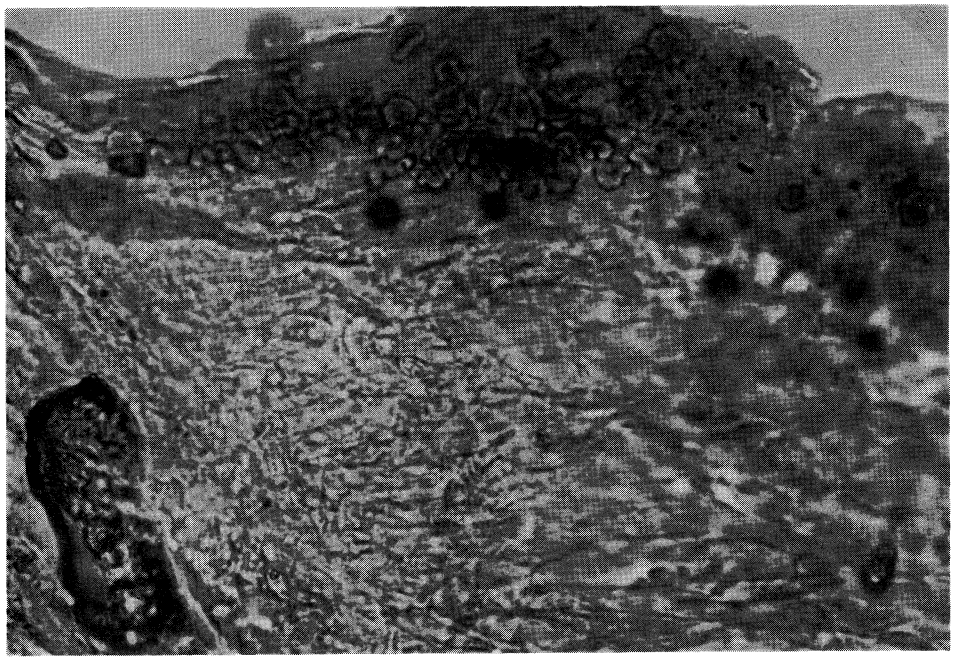

Fig. 4. E_N rosette formation in guinea pig epithelial tissue on day 4 after grafting. (Giemsa stain ×400)

T lymphocytes in human lymphoid tisses could be identified in lymphnode, thymus, spleen[3,10] and spleen,[9] but not in lymphnode, thymus, spleen[7] and lymphnode[4,5,8] with sheep erythrocyte rosettes. In our study, the E_N rosettes were formed in splenic white pulp, but not so many. Stingle *et al.*[8] reported that the rosette formation depends on the cell viability and only living T lymphocytes can be detected. However, Wilson *et al.*[11] reported that rabbit erythrocytes reacted with both live and dead T lymphocytes despite of the decreased numbers of the formed rosettes with the latter cells. Brubaker *et al.*[6] considered the time of incubation wilh erythrocytes as one of the paramaters of the successful rosetting and claimed that an incubation no longer than 45 min was unsuitable. In our study, the rosettes were formed finely with the incubation for 5 hrs at 4°C. Longer incubation was avoided, for the overnight incubation at 4°C resulted in the autolysis of the tissue component in some cases.[10]

Neuraminidase appears to increase the binding capacity of erythrocytes to lymphocytes.[2] In our study, it is comfirmed that the combination of neuraminidase treatment of erythrocytes and the humidity chamber technique is a simple and useful method for the demonstration of T lymphocytes in guinea

pig tissue sections. Bjerke *et al.*[9] treated the specimens with DAB before haematoxilin and eosin staining to enhance the colour contrast. Applying this reagent to our specimens, the rosettes were fairly clear as shown in Fig. 3.

The localization of T lymphocytes in the allografted skin is observed by the modified rosette forming assay described here. In addition, the scanning electron microscopic observation displayed the characteristic adherence of rabbit E_N to guinea pig blood T lymphocyte in suspention. If using both this rosette forming assay and SEM, the adherence of rabbit E_N to guinea pig T lymphocytes in frozen tissue sections could be observed in more detail.

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