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Detection of tissue T-cell in patients with chronic active hepatitis using fragmented sheep red blood cell (SRBC) membrane.

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Abstract

Fragmented sheep red blood cell (SRBC) membrane was used for detection of T-cells in liver biopsy specimens from patients with chronic active hepatitis. SRBC was separated with Lymphoprep, sonicated, then filtered through a 3 μ Millipore-membrane as a fragmented SRBC reagent. Tissue T-cells were stained by an indirect immunofluorescent technique using SRBC reagent and fluorescein isothiocyanate (FITC)-labelled rabbit anti-SRBC. Positively staining lymphocytes were present in portal tracts and in areas of piecemeal necrosis. There also seemed to be a positive correlation between the number of positively staining lymphocytes and the activity of chronic hepatitis; numerous lymphocytes being stained in areas of severe piecemeal necrosis. Our findings suggest that the fragmented SRBC technique for detection of T-cells is reliable and reproducible, that it could be used as a clinical routine method, and that it is useful for further elucidating the nature of host immune reactions on tissue levels.

KEYWORDS: T-lymphocyte, T-cell, lymphocyte receptor, chronic active hepatitis, immune response.

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**DETECTION OF TISSUE T-CELL IN PATIENTS WITH
CHRONIC ACTIVE HEPATITIS USING FRAGMENTED
SHEEP RED BLOOD CELL (SRBC) MEMBRANE**

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Abstract. Fragmented sheep red blood cell (SRBC) membrane was used for detection of T-cells in liver biopsy specimens from patients with chronic active hepatitis. SRBC was separated with Lymphoprep, sonicated, then filtered through a 3 μ Millipore-membrane as a fragmented SRBC reagent. Tissue T-cells were stained by an indirect immunofluorescent technique using SRBC reagent and fluorescein isothiocyanate (FITC)-labelled rabbit anti-SRBC. Positively staining lymphocytes were present in portal tracts and in areas of piecemeal necrosis. There also seemed to be a positive correlation between the number of positively staining lymphocytes and the activity of chronic hepatitis; numerous lymphocytes being stained in areas of severe piecemeal necrosis. Our findings suggest that the fragmented SRBC technique for detection of T-cells is reliable and reproducible, that it could be used as a clinical routine method, and that it is useful for further elucidating the nature of host immune reactions on tissue levels.

Key words: T-lymphocyte, T-cell, lymphocyte receptor, chronic active hepatitis, immune response.

The host immune response to hepatitis virus associated antigens (1-15) and liver specific membrane antigens (16-24) has been reported as the most important mechanism in pathogenesis of hepatic cell necrosis in patients with chronic active hepatitis.

For the analysis of the host immune response, the function of T- and B-lymphocytes in the peripheral blood of such patients has been examined by the technique of rosette formation with sheep red blood cells (SRBC) (25-30). It is unlikely that studying lymphocytes in peripheral blood will give any data on the lymphocyte localization pattern in liver tissue. Furthermore, the use of SRBC

as a large marker on tissue levels probably will not give resolution good enough for detailed information on the distribution on lymphocytes (31-34).

In the present paper, the localization of T-cells in liver tissue of patients with chronic active hepatitis was examined by an indirect immunofluorescent technique, with finely fragmented SRBC membrane used as the first antiserum and FITC-labelled anti-SRBC as the second antiserum.

MATERIALS AND METHODS

The preparation of fragmented SRBC membranes is summarized in Fig. 1. Twenty ml of sheep whole blood was centrifuged at 20°C for 30 min at 400 rpm.

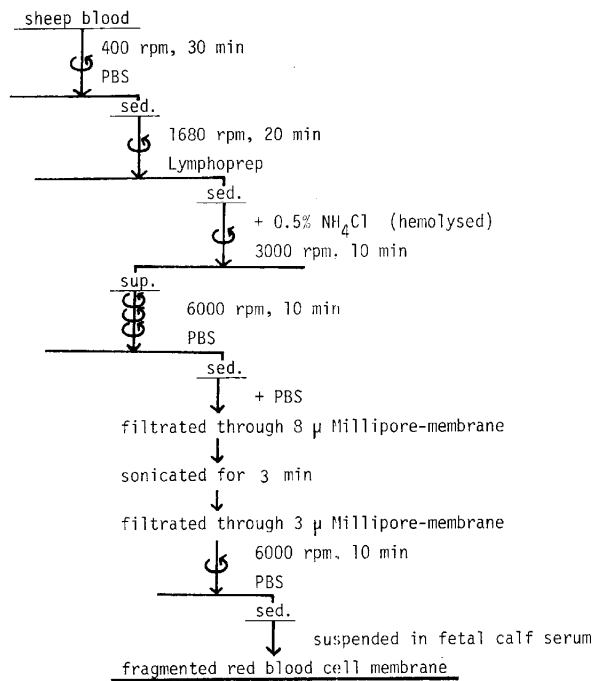


Fig. 1. Separation of sheep red blood cell (SRBC) membranes

The sediment containing a concentrate of red blood cells (RBC) was washed 3 times with physiological saline solution buffered with 0.005 M phosphate to pH 7.4 (PBS). The RBC-preparation was diluted with one volume of minimal essential medium (MEM) (Gibco) containing 25 mM Tris (pH 7.4) at 20°C and 1 ml was layered on top of 3 ml Lymphoprep (sodium metrizoated/Ficoll solution: Nyegaard & Co.) of specific gravity 1.077 present in each of 5 tubes with a diameter of 14mm. After centrifugation at 20°C for 20 min at 1,680 rpm, RBC were resuspended in 3 ml of an ice-cold isotonic NH₄Cl, 10mM KHCO₃ and 0.1mM

EDTA (pH 7.4) to accomplish lysis of RBC. Hemolysed SRBC were centrifuged for 10 min at 6,000 rpm, and resuspended in 5.0 ml PBS. The suspension was filtrated through 8 μ Millipore-membrane (Millipore Co.). The filtrated SRBC were sonicated for 30 min, filtrated through 3 μ Millipore-membrane, and centrifuged for 10 min at 6,000 rpm. The pellet was resuspended in 10 ml of fetal calf serum, and was used as the final preparation of fragmented SRBC membranes.

FITC-labelled rabbit anti-SRBC was prepared using "Hemolysin" (Kyokuto Co.) and fluorescein isothiocyanate Isomer I (FITC) (BBL). The γ -globulin of antibody to SRBC was separated by precipitation with saturated ammonium sulfate with FITC at 4°C for 6 h. The solution of conjugate was eluted on a Sephadex G-25 course column and a DEAE-cellulose column. The ratio of protein/FITC was 1/1.5-1.6.

Cryostat non-fixed liver sections from 2 cases of severe chronic aggressive hepatitis (CAH) (36), 2 cases of moderate CAH and one healthy person, were stained with the fragmented SRBC and FITC-labelled anti-SRBC. The concentration of fragmented SRBC used was 0.1 mg protein/ml as measured by the modified micro-Ouchterlony method previously reported (37). The titer of FITC-labelled anti-SRBC was 32 times that of the fragmented SRBC membrane antigen.

The percentage of lymphocytes coated with fragmented SRBC membranes was determined after counting 250-300 lymphocytes by two observers who had no knowledge of the identity of the material. Only lymphocytes fixed with at least three fragmented SRBC membranes were scored as fragmented-SRBC coated lymphocyte.

RESULTS

Relationship between peripheral E-rosette forming lymphocytes (T-cells) and peripheral lymphocytes coated with fragmented SRBC. The technique of E-rosette formation and of coating with fragmented SRBC were applied to the peripheral lymphocytes of various cases of CAH and the results are summarized in Table 1.

TABLE 1. E-ROSETTE-FORMING-LYMPHOCYTES AND LYMPHOCYTES COATED WITH FRAGMENTED SHEEP RED BLOOD CELL (SRBC) IN THE PERIPHERAL BLOOD OF VARIOUS PATIENTS

Case No.	Diagnosis ^a	Age/Sex	Serum HBsAg	No. of E-rosette lymphocyte (T-cell)/No. of total lymphocyte (%)	No. of lymphocyte with fragmented SRBC/No. of total lymphocyte (%)
1	CAH (2B)	28/male	+	105/300 (35.0)	98/240 (40.8)
2	CAH (2B)	34/female	-	116/270 (43.0)	113/250 (45.2)
3	CAH (2A)	24/male	+	140/300 (46.7)	178/300 (59.3)
4	CHA (2A)	42/male	-	144/270 (53.3)	156/250 (62.4)
5	Healthy	30/male	-	205/300 (68.3)	218/300 (72.7)

^a: CAH (2B)=Severe chronic aggressive hepatitis, CAH (2A)=mild chronic aggressive hepatitis.

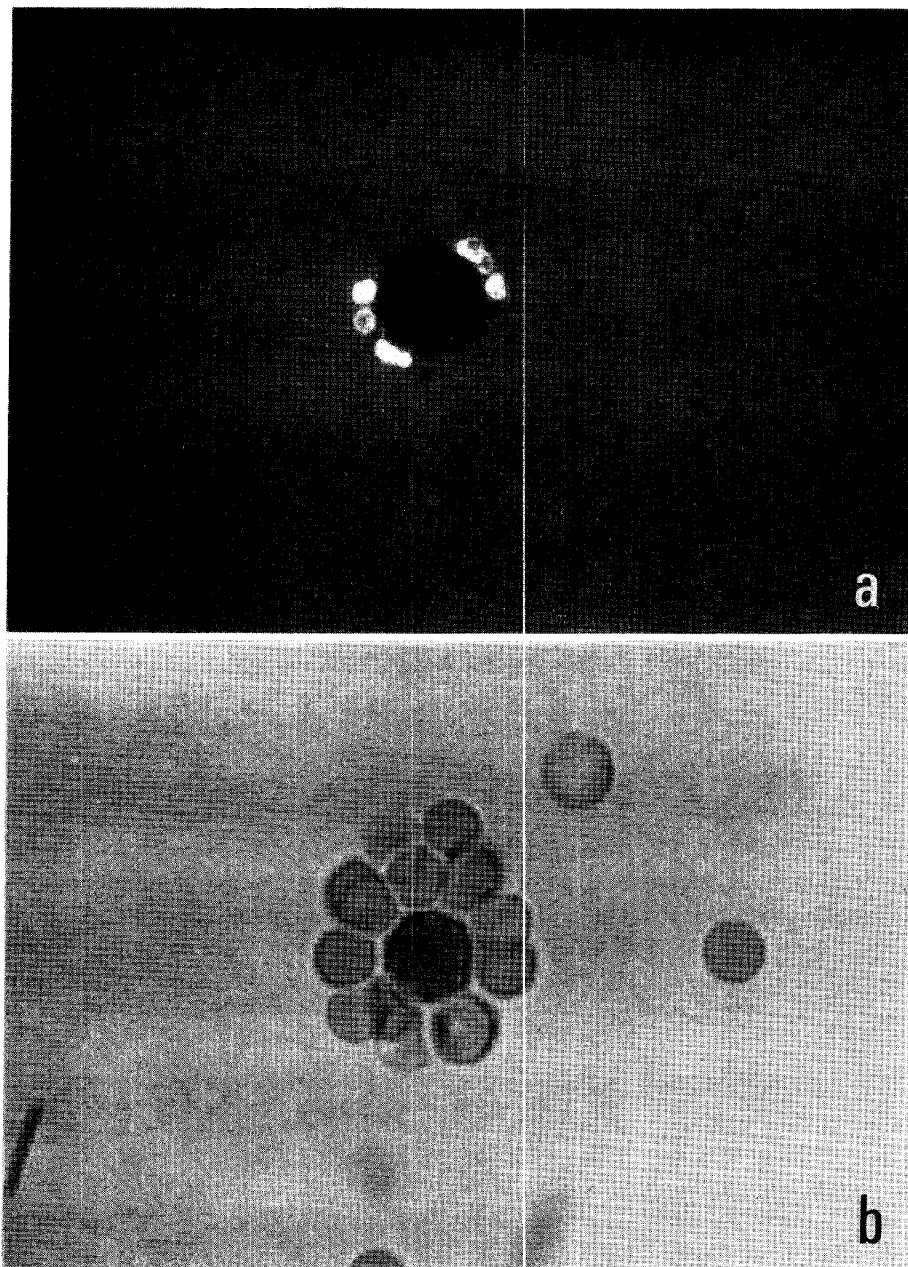


Fig. 2. T-cells in peripheral blood from a patient with moderate CAH by two methods.
a) A lymphocyte coated with fragmented sheep red blood cell (SRBC) membranes.
×1,000.
b) An E-rosette forming lymphocyte as previously reported by Yata and Tachibana.
×800.

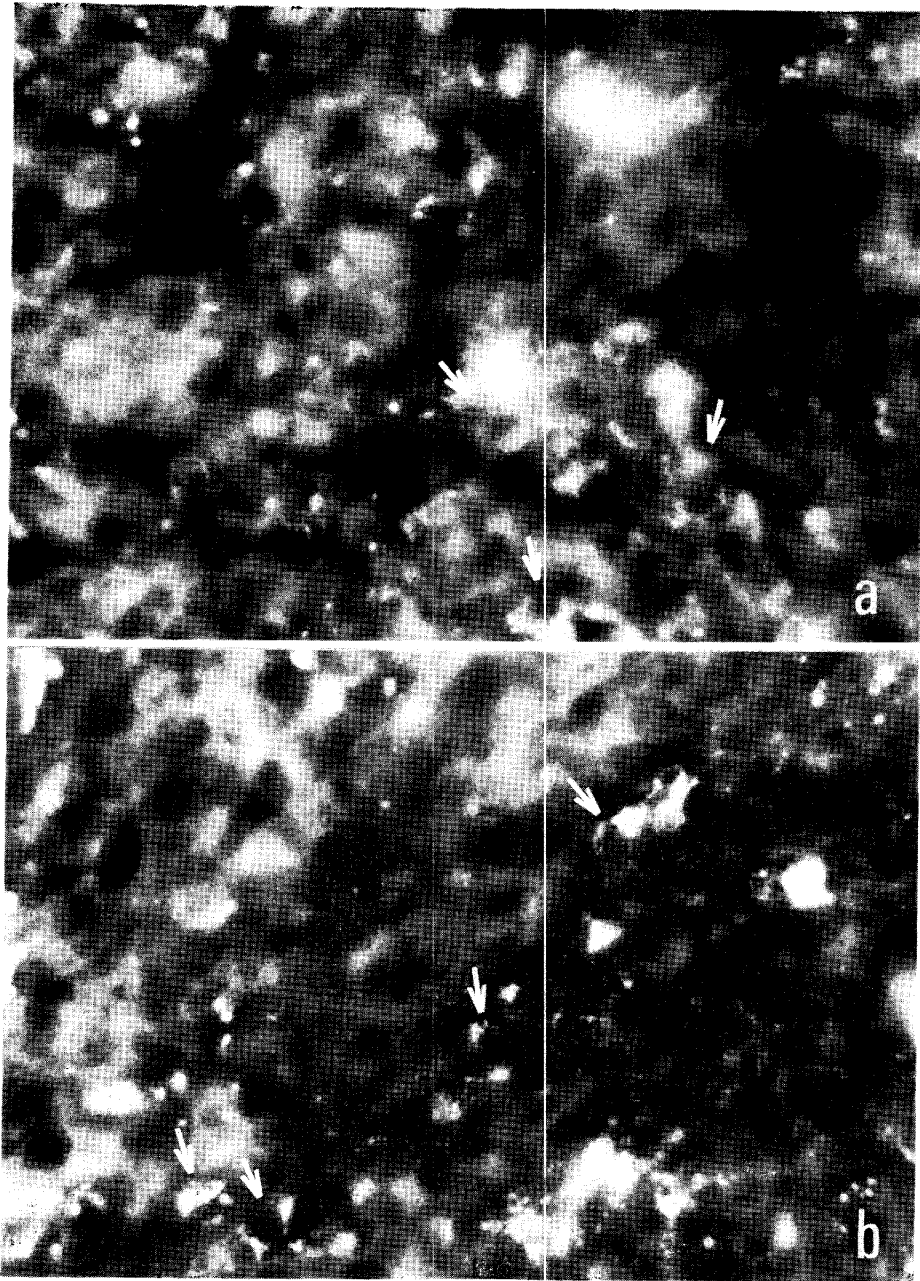


Fig. 3. Localization of T-cells in liver section from a patient with severe CAH.

- a) Lymphocytes coated with fragmented SRBC (↓) are shown in an area of piecemeal necrosis where many lymphocytes are observed. $\times 400$.
- b) Lymphocytes coated with fragmented SRBC (↓) are shown in the portal tract area without any clear attachment to hepatic cells. $\times 400$.

T-cells in severe CAH were 35.0 and 43.0% and lymphocytes coated with fragmented SRBC were 40.8 and 45.2%. In moderate CAH, T-cells were 46.7 and 53.3% and fragmented-SRBC-coated lymphocytes were 59.3 and 62.4%. In a healthy person, T-cells were 68.3% and fragmented-SRBC-coated lymphocytes were 72.2%. The patterns of peripheral lymphocytes coated with fragmented SRBC and rosette formation T-cell are shown in Fig. 2.

Results of tissue T-cells in patients with various chronic hepatitis. Lymphocytes coated with fragmented SRBC in liver tissue were detected among the lymphocytes present in areas of piecemeal necrosis and portal tracts where many lymphocytes were observed (Fig. 3-a). Positively staining lymphocytes were also seen in areas of focal necrosis in liver acini and in sinusoidal veins. In all cases of CAH patients, positively stained lymphocytes in piecemeal necrosis areas were separated from intact hepatic cells (Fig. 3-b). In regard to relationship between severe CAH and moderate CAH, positively stained lymphocytes were detected more in severe cases than in moderate cases (Figs. 3, 4).

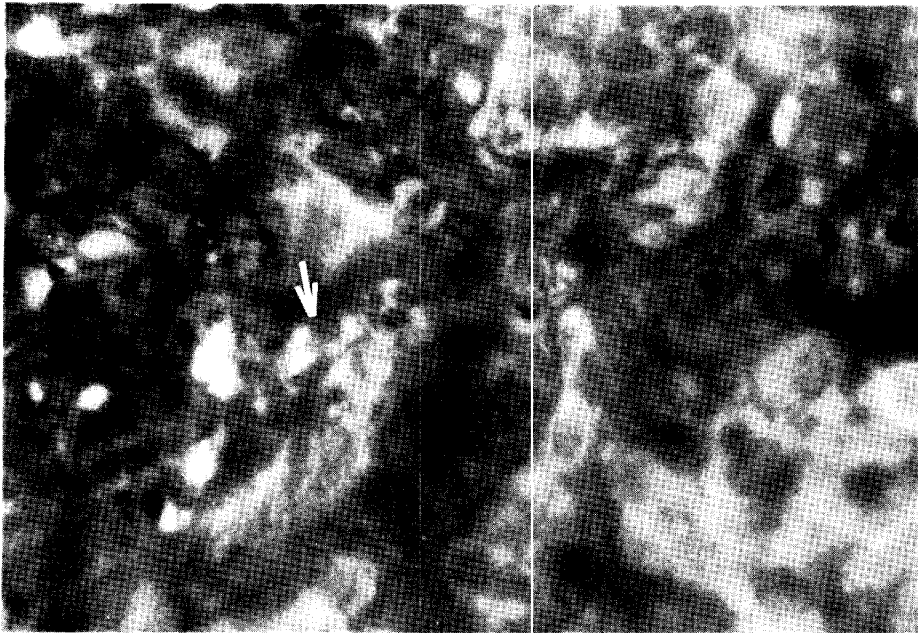


Fig. 4. Localization of T-cells in liver from a patient with mild CAH. A lymphocyte coated with fragmented SRBC ($\downarrow$) is shown in an area of the portal tract. $\times 400$.

DISCUSSION

The sensitivity of SRBC (E) rosette forming lymphocytes has already been reported to be lower than that of SRBC coated with antibody IgG and comple-

ment (EAC) (33, 34, 40-46). The authors thought this was due to the energy for binding E cells to lymphocytes being less than that of EAC cells. In the present paper, the fragmented SRBC membrane was clearly detected on the membrane of lymphocytes. The sensitivity of the fragmented SRBC method was higher than that of the E-rosette forming technique, but the results for peripheral lymphocyte were similar for both methods.

In liver tissue, fragmented SRBC coated lymphocytes were detected on the membrane of lymphocytes without any clear attachment to hepatic cells in areas of piecemeal necrosis. The function of T-cells and their significance in the development of tissue damage in chronic active hepatitis is not clear, but T-cells may be involved in the host immune response to hepatitis virus. The fragmented SRBC technique presented here for the detection of tissue T-cells is sensitive and specific, and seems to be useful for the study of the role of T-cells in hepatitis and other diseases.

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