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Author(s)	ONOÉ, Kazunori; YASUMIZU, Ryoji; TAKEDA, Junko; OKUYAMA, Harue; MORIKAWA, Kazuo
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## The Study of Difference in the Proportions of FcR<sup>+</sup> Lymphocytes between the Spleen and the Lymph Node

Kazunori ONOÉ, Ryoji YASUMIZU, Junko TAKEDA,  
Harue OKUYAMA and Kazuo MORIKAWA

*Division of Pathology, Institute of Immunological Science,  
Hokkaido University, Sapporo, Japan*

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**Abstract** The surface receptors for rabbit erythrocyte (E), sheep E-antibody complex (EA) and sheep E-antibody-complement complex (EAC) in guinea pig lymphoid cell were investigated using rosette assay.

Forty one per cent of lymph node cells and 32 per cent of spleen cells bound E. Though significant number (42 per cent) of spleen cells bound EA, only a few percentage (5 per cent) of lymph node cells did. No difference in number of EAC binding cells between the spleen and the lymph node (24 per cent) were observed.

It is discussed why most lymph node cells don't bear FcR identified with an EA rosette technique in spite of significant number of these cells in the spleen.

Several groups have shown receptors for the Fc portion of IgG (Fc) on various cell types and investigated the function of the cells carrying it. The methods to study FcR on cell membrane have generally utilized rosette formation and fluoresceinated or radiolabeled soluble complex binding. These techniques, however, were with different sensitivities for detection of FcR and the proportions of FcR<sup>+</sup> cells reported in these studies displayed apparent discrepancy.

We used EA (sheep erythrocyte (E)-rabbit anti-sheep E IgG) rosette to detect FcR<sup>+</sup> lymphocytes, EAC (sheep E-rabbit anti-sheep E IgM-AKR mouse complement) rosette for complement receptor lymphocytes (CRL) and rabbit E spontaneous rosette for T cells in the lymph node, spleen, thymus and peripheral blood lymphocytes (PBL).

Hartley/M, Strain 2 and Strain 13 guinea pigs were used in this investigation. Rosette assay with lymphoid cell suspensions were performed as previously described<sup>13,15,19</sup>, with an exception of EA rosette assay partly modified. Briefly, sheep erythrocytes were sensitized with a subagglutinating dose (1/200) of heat inactivated, hyperimmune rabbit anti-sheep E IgG and washed three times in cold phosphate buffered saline (PBS).  $3 \times 10^7$  of EA were mixed with  $3 \times 10^6$  lymphoid cells in a total volume of 0.6 ml Hanks' solution and incubated at 37 °C for 5 minutes. These mixed cell suspensions were then centrifuged at 800 rpm for 5 minutes at 4 °C. After overnight incubation at 4 °C the pellet was gently resuspended and 0.1 ml of rosette preparation was mixed with 0.1 ml of crystal violet solution (PBS containing 1 per cent glutaraldehyde and 2 per cent crystal violet). Any stained cell which bound three or more EA was considered as a rosette under light microscope. The results were expressed as the percentage of rosette in relation to the total number of nucleated cells. Burst cells and the cells apparently identified as non-lymphoid cells from the morphological view were excluded from the counting.

A significant number of EA rosette forming cells (EA RFC) were found in the PBL (13 per cent) and the spleen (42 per cent). In contrast, very few EA RFC were found in the lymph node

(5 per cent) and the thymus (2 per cent) (Fig. 1).

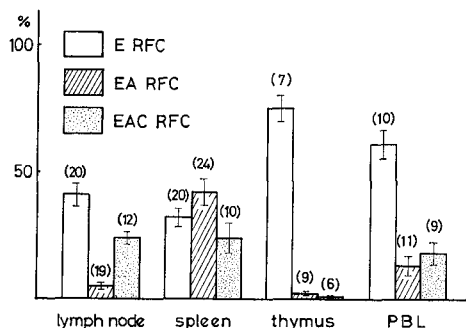
CRL were 24% both in the spleen and in the lymph node, whereas 42 and 5 per cent EA RFC were found in the former and the latter, respectively. Only a few macrophages detected by peroxidase staining and a few IgM-EA RFC were in the spleen cell suspension (Table 1). It is generally considered that FcR<sup>+</sup> cells detected by EA rosette assay are B cells<sup>12</sup>. If it is true, it is presumed that about 20 per cent of B cells in the spleen carrying FcR but lacking CR and that almost the same percentage of B cells in the lymph node carrying CR but lacking FcR.

After cell filtration through a nylon wool column, a procedure known to remove B cells and monocytes, spleen and lymph node T cell proportions increased approximately 35 per cent, but EA RFC in the spleen decreased only 14 per cent and there were few EA RFC in the lymph node cells (Fig. 2). Accordingly there were no T cells which bound EA in the lymph node but some of EA RFC remaining in the spleen cell suspensions after nylon wool filtration might belong to a T cell subset even if taking into the consideration that these suspensions contained T cells only at 64 per cent.

Spleen cell suspension was mixed simultaneously with EA and rabbit E and tested for the rosette formation by procedures as described in the EA rosette formation. As rabbit E are much larger than sheep E, E RFC could easily be distinguished from EA RFC. The proportion of mixed E-EA RFC was about 4 per cent of the whole spleen cell suspension and 24 per cent of E RFC (Table 2). The results clearly show that FcR<sup>+</sup> T cells as well as FcR<sup>+</sup> B cells exist in the spleen.

It has been generally accepted that there were significant numbers of FcR<sup>+</sup> lymphocytes in the spleen, bone marrow and PBL. However, the results in the lymph node, thymus and thoracic duct lymphocytes (TDL) were contradictory<sup>9</sup>. The difference of techniques and animal species used could probably influence with ease in demonstrating FcR on different cell types. Most investigators who used EA rosette assay reported that there were virtually no FcR<sup>+</sup> cells in the thymus, lymph node and TDL<sup>8,10,18</sup>.

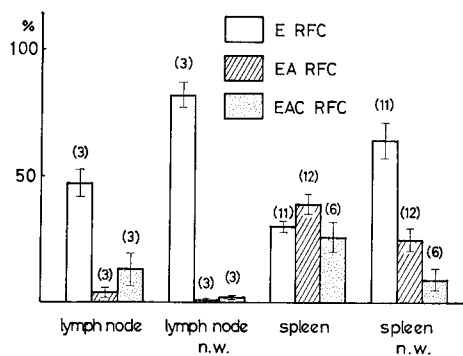
We introduced an overnight incubation at 4°C in EA rosette assay into the experiment and could show relatively constant results. No FcR<sup>+</sup> T cells and only a few B cells were observed in the lymph node. This result was coincident with the reports mentioned above.



**Fig. 1.** The frequency of lymphocytes (%) with E, EA and EAC rosettes in the thymus, lymph node, spleen and PBL suspensions. Values indicate the mean  $\pm$  S.E. and numbers of animals are indicated in parentheses.

**Table 1.** Proportions of IgG EA, IgM EA RFC and peroxidase positive cells in the spleen and lymph node cell suspensions. IgM EA rosette assay was performed as described in the text except that anti-sheep E IgM (1/30) was used instead of IgG. Peroxidase staining was done using 2, 7-Fluorenediamine. Numbers of animals are indicated in parentheses.

	Spleen (%)	lymph node
IgG EA	42.0 $\pm$ 1.4 (2)	ND
IgM EA	2.0 $\pm$ 1.4 (2)	ND
peroxidase (+) cells	2.43 $\pm$ 0.42 (3)	0.07 $\pm$ 0.12 (3)



**Fig. 2.** The percentage of E, EA and EAC RFC in the lymph node and the spleen cell suspensions after nylon wool column filtration. The nylon wool column filtration was performed as previously described<sup>11</sup>. (n.w.: nylon wool effluent cells)

thymocytes were essentially not reactive. Effector cells in ADCC are known as FcR<sup>+</sup> lymphoid cells<sup>6</sup>.

Diamond et al.<sup>3</sup> showed that lymph node lymphocytes respond slightly better to PHA than spleen cells while Con A was a more efficient mitogen for spleen cells than lymph node cells. Stout and Herzenberg<sup>20</sup> reported that the response to Con A was found to be a characteristic of FcR<sup>+</sup> T cells.

Furthermore, in investigating the circulatory behaviour of lymphocytes, Diamond et al.<sup>3</sup> observed that lymph node seeking cells migrated predominantly to the lymph node whereas spleen seeking cells returned to the spleen. Flad et al.<sup>5</sup> reported that B cells in chronic lymphocytic leukemia (CLL) could not recirculate from blood to lymph. Most of CLL B cells were generally considered as FcR positive<sup>4</sup>.

Lymphocytes enter the lymph nodes in large numbers because of their affinity for a specialized endothelium in post capillary venule (PCV) situated in a deep zone of the lymph node cortex<sup>9,21</sup>. But surface determinants of recirculating lymphocytes essential for homing into the lymph nodes have not been determined as yet<sup>7,22</sup>. While lymphocytes can enter the spleen and bone marrow directly from blood circulation, almost no cell can migrate into the lymph node and thoracic duct without passing through PCV with exception of the afferent drainage.

Thus, the great differences in the proportions of FcR<sup>+</sup> cells between the former and the latter tissue suspensions can be easily accounted for, if it is assumed that only FcR<sup>-</sup> lymphocytes migrate into the endothelium of PCV. This hypothesis may also explain the reason why monocytes and granulocytes bearing FcR can not be observed in the wall of PCV and the parenchyma of the lymph node in the normal condition.

Five per cent FcR<sup>+</sup> cells in the lymph nodes may enter via afferent lymphatics. The proportion of FcR<sup>+</sup> cells in the regional lymph node increased from  $5.5 \pm 2.8$  per cent to  $9.6 \pm 2.9$  and  $13.7 \pm 4.7$  per cent, 4-5 and 18-24 days respectively after administration of oxazolone with complete Freund adjuvant<sup>16</sup>. These increases are probably owing to the influx of FcR<sup>+</sup> cells via afferent lymphatics draining the region.

Approximately 43 per cent of FcR<sup>+</sup> lymphocytes in the spleen lacked CR. Though a part of

**Table 2.** Proportions of E, EA and E-EA double RFC. Spleen cell suspension was mixed with E and EA simultaneously. The right column express the proportion of E-EA double positive RFC divided by total E RFC.

Exp. No.	E RFC (%)	EA RFC (%)	E-EA RFC (%)	E-EA RFC/E RFC (%)
89	13	48	3	20
90	19	53	5	28
91	18	58	4	24
average	16.7	53.0	4.0	24.0

Recently, O'Toole et al.<sup>17</sup> and Revillard et al.<sup>18</sup> showed in antibody dependent cell-mediated cytotoxicity (ADCC) assay system that spleen cells and PBL were highly reactive, but lymph node cells and

these cells were evidently T cells (Table 2), majority might be identical with surface Ig<sup>-</sup>, FcR<sup>+</sup> lymphocytes showed by Abo et al.<sup>1)</sup> or L cells reported by Lobo and Horwitz<sup>10)</sup> in human PBL.

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