

## DYNAMIC ANALYSIS OF LYMPHOCYTE MIGRATION INTO PEYER'S PATCHES OF RAT SMALL INTESTINE

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### ABSTRACT

*Dynamic aspects of lymphocyte immigration into Peyer's patches were analyzed in rat small intestine using intravital fluorescence microscopy. Lymphocytes from intestine, lymph, and spleen were labeled with acridine orange in vitro and infused from the mesenteric artery of rats. There are three types of lymphocytes as characterized from their interaction with post-capillary venules of Peyer's patches immediately after infusion, and most of the lymphocytes passed through the microcirculatory bed without any interaction with venules. About 15 to 20 minutes later, the number of lymphocytes which stick to the Peyer's patches was increased and reached a maximum at 40 to 50 minutes. There was a difference in distribution pattern of infused lymphocytes between spleen cells and lymphocytes from intestinal lymph suggesting the complex control mechanism of lymphocyte migration into Peyer's patches.*

Mesenteric lymphatics are the major traffic pathway of recirculating lymphocytes. This constant traffic of lymphocytes may play a role in the cell-cell interaction necessary for functioning of the immune defense system in the intestinal wall (1). Peyer's patches have no afferent lymphatic vessels and lymphocytes are known to migrate into Peyer's patches

through the post-capillary venules (2). Homing specificity of lymphocytes has been considered to be determined by the specific interaction between recirculating lymphocytes and post-capillary high endothelial venules (3). These continuous lymphocyte streams through Peyer's patches are essential for function as secondary lymphoid organs initiating immune reactions. However, there is a comparative paucity of data about the dynamic aspect of lymphocyte traffic through Peyer's patches (4). In this study, therefore, the course of lymphocyte immigration into Peyer's patches was analyzed by intravital fluorescence microscope to clarify how lymphocyte immigration occurs after the interaction of immigrated lymphocytes with the venules of patches.

### MATERIALS AND METHODS

Male Wistar rats about 250g were used throughout the experiment. Spleen cells were separated by the method of Butcher and Weissman (5) and lymphocytes from intestinal lymph were obtained by the method of Bollman et. al. (6). Single cell suspensions of lymphocytes were labeled with acridine orange (2 $\mu$ l added per 3 $\times 10^6$  cells) for one minute in RPMI medium. After repeated washing, 1 $\times 10^7$  cells in 1ml suspension medium were prepared. Under intraperitoneal pentobarbital anesthesia, an ileal loop

near the cecum was gently pulled out and spread over a glass plate equipped with a small water bath which was maintained at 37°C. The intestine containing Peyer's patches was covered with a cover glass and observed *in vivo* from the serosal side of the intestine under a fluorescence microscope equipped with silicon-intensified target video camera (Hamamatsu Photonics, Japan). Spleen cells or intestinal lymph cells ( $1 \times 10^7$ ) were injected into the mesenteric artery of rats through indwelling 27 gauge needles. The cell kinetics of infused lymphocytes and their immigration into Peyer's patches were monitored and recorded on U-matic video tapes.

## RESULTS

The cell kinetics of infused cells were divided into three types according to their interaction with postcapillary venules of Peyer's patches immediately after the infusion of spleen lymphocytes (within 40 seconds) (*Fig. 1*). The total



*Fig. 1. Peyer's patch of rat small intestine immediately after the infusion of spleen lymphocytes labeled with acridine orange. Most of the infused lymphocytes are passing through microvascular beds while some of them are attached to the Peyer's patch.*

number of lymphocytes appeared in the video frame at the time of infusion was  $723 \pm 78$ . As shown in *Fig. 2*, most of the lymphocytes ( $82.5 \pm 2.1\%$ ) simply passed through the microcirculatory bed without an interaction with the venules. Among these passed-through lymphocytes, 72% were very rapidly cleared from the frame, while 10.6% of the cells were

slowly passed away after rolling along the venular walls. Some lymphocytes ( $14.7 \pm 2.4\%$ ) attached to the venular walls, detached soon after. Among these transient sticking lymphocytes, most of them ( $11.1 \pm 1.9\%$ ) were detached rapidly within one second, but there were certain lymphocytes ( $1.0 \pm 0.2\%$ ) which took more than 5 seconds to detach from the venules. Only  $1.2 \pm 0.3\%$  of total spleen lymphocytes were "stuck" and remained inside Peyer's patch after the infusion of lymphocytes from the mesenteric artery at this period (within 40 seconds). There were no apparent differences between spleen cells and lymphocytes from intestinal lymph in the lymphocyte interaction pattern to venular walls immediately after the infusion of lymphocytes.

Soon after the infusion of lymphocytes, the sticking cells in Peyer's patches were gradually increased. The recirculating lymphocytes eventually migrated into Peyer's patches (*Fig. 3*). The time course of homing of spleen lymphocytes in the determined area ( $12\text{mm}^2$ ) of Peyer's patch is shown in *Fig. 4*. There were not many lymphocytes sticking in the determined area within 10 minutes. About 20 minutes after the infusion of lymphocytes, the number of sticking lymphocytes to the venules was significantly increased. At this time, the major part of cells were seen inside the venules (about 63%) and few lymphocytes were seen inside follicles apart from the venules. It is also noteworthy that at this time, most of cells inside and along the venules were stuck to the larger size (more than  $25\mu\text{m}$ ) venules in the case of spleen cell infusion. The number of lymphocytes homing to Peyer's patches reached their maximum at 40 to 50 minutes after the infusion and at that time the distribution of labeled lymphocytes was different from that of the earlier period. Namely, the percentage of lymphocytes inside the venules was decreased, and instead, the lymphocytes along venules and inside follicles were increased. Moreover, at 50 minutes, the number of lymphocytes stuck inside and along the smaller venules (less than

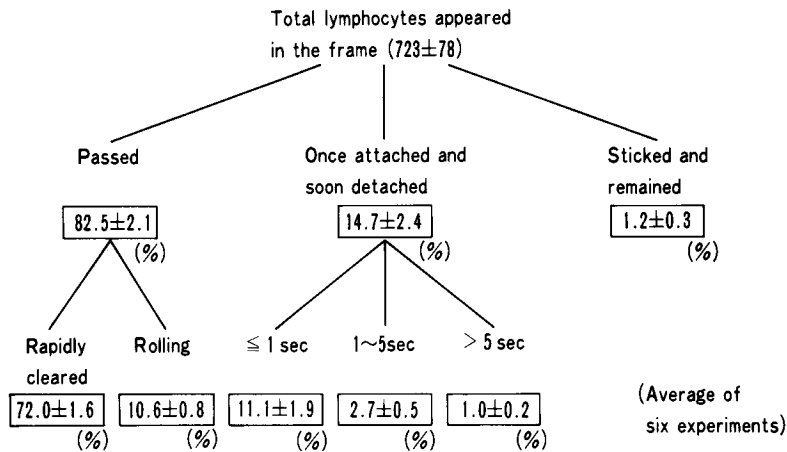


Fig. 2. Interaction of spleen lymphocytes with venular walls of Peyer's patches immediately after infusion.

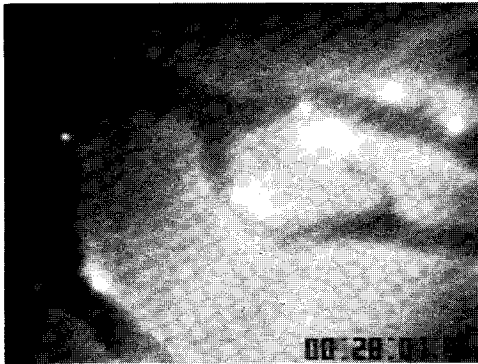


Fig. 3. About 23 minutes after the infusion of spleen lymphocytes. Sticking cells inside and along venules of Peyer's patch were gradually increased.

25 $\mu$ m) was greater than that of larger venules.

When lymphocytes from intestinal lymph (T-cell 75%) were infused, the time course of distribution pattern of infused lymphocytes was different from those of the spleen cells (B-cell 62%). The total number of cells residing in the area was smaller than that of spleen cells. These cells from intestinal lymph tended to stick to the endothelium of the smaller post-capillary venules (>25 $\mu$ m) even at 20 to 30 minutes after the infusion. Also cells from intestinal lymph were shown to be transported inside the follicles earlier than the spleen cells.

## DISCUSSION

These results suggest that it takes more than 10 minutes for recirculating lymphocytes to gain residence into Peyer's patches. Lymphocyte migration into Peyer's patches may not be completed by a single interaction, but rather by the repeated encounter of recirculating lymphocytes to the post-capillary venules of Peyer's patches. There are differences in distribution site and time course of infused lymphocytes between spleen cells and lymphocytes from intestinal lymph. Organ specificity of lymphocyte migration mediated by highly selective lymphocyte interaction with organ-specific determinants on high endothelial venules has been postulated by Butcher et. al. (7). However, Stevens et. al. (8) recently postulated that the homing characteristics of B and T cell populations are largely independent of their organ origin, and B cells from any source distribute preferentially to Peyer's patches, whereas T cells home preferentially to peripheral lymph nodes. These data are consistent with our results that lymphocytes from spleen cells (B cell rich) tend to stick to post-capillary venules in greater number than those from intestinal lymph (T cell rich). A specific recognition structure on lymphocytes might play a role in recognition and binding to Peyer's patches. Chin et.

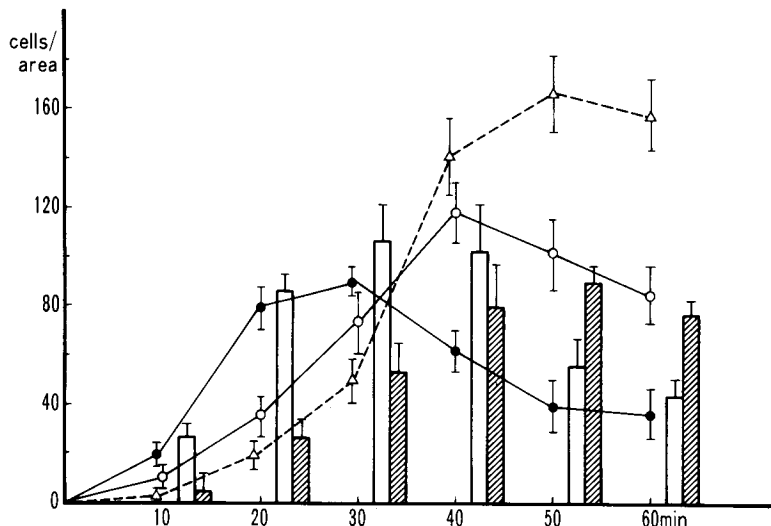


Fig. 4. Time course of homing of spleen lymphocytes in the determined area ( $12\text{mm}^2$ ) of Peyer's patch. Mean  $\pm$  SEM ( $n=5$ ),  $\bullet$ — $\bullet$  inside venules,  $\circ$ — $\circ$  along venules,  $\Delta$ — $\Delta$  inside follicles,  $\square$  lymphocytes in and along venules of larger size ( $>25\mu\text{m}$ ),  $\text{hatched}$  lymphocytes in and along venules of smaller size ( $\leq 25\mu\text{m}$ ).

al. (9) have demonstrated that rat lymph contains two antigenically distinct high endothelial binding factors that exhibit an *in vitro* affinity for lymphocyte-binding site of either high endothelial venules of lymph nodes or Peyer's patches. Based on our observations, it is possible that there is a difference in the receptor of high endothelial venules between larger venules and smaller venules even in the same Peyer's patches. The changes of the distribution pattern of sticking lymphocytes in Peyer's patches suggest that the binding of these lymphocytes to the specific receptor is not only involved in lymphocyte immigration but is also important in the phase of settlement of lymphocytes inside follicles. Further investigations are necessary to clarify the complex control mechanism of lymphocyte migration into Peyer's patches.

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