Presence of natural killer cells in Peyer's patches of the mouse[1]

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Summary. Natural cell-mediated cytotoxicity of normal murine Peyer's patch cells against Ehrlich ascites carcinoma was found in a short-term ^{51}Cr release assay. Peyer's patch and lymph node cells showed natural cytotoxicity at approximately the same level.

Peyer's patches are an important constituent of gut-associated lymphoid tissue. Both T and B lymphocytes are present in this organ[2,3]. The participation of Peyer's patches in primary humoral[4] and cellular[5-7] immunity is rather weak. Macrophages are scarce in Peyer's patches and the concentration of suppressor cells in this organ is high[8,9]. The immunological role of Peyer's patches is not completely understood.

Natural cell-mediated cytotoxicity is suggested to be an important factor in host resistance against cancer. The presence of natural killer (NK) cells was detected in lymphoid organs of man, mouse, rat and guinea-pig[10-13], as tested against a variety of iso-, allo-, and xenogeneic tumor target cells[12,14,15].

The aim of the present study was to investigate whether NK cells are also present in Peyer's patches of mice.

Materials and methods. Inbred female CFW/L1, 6-10-week-old mice were used. Spleen, peripheral lymph nodes (axillary and inguinal), mesenteric lymph nodes, thymus and Peyer's patches were dissected out from untreated donor mice and were cut into fine pieces with scissors in cold Eagle's minimal essential medium (MEM). They were then squeezed through an 80 stainless steel mesh, washed twice with cold MEM and filtered through a 200 stainless steel mesh. The number of cells was counted; their viability ranged between 91 and 95% as estimated in a Trypan blue exclusion test. For each experiment lymphoid organs from 3 mice were pooled.

Ehrlich ascites carcinoma cells (EAC), maintained in CFW/L1 mice, were used as target tumor cells. EAC cells were aspirated from the peritoneal cavity of mice injected 6-8 days earlier with tumor cells. The cells were washed twice in MEM and filtered through a 200 stainless steel mesh.

The cell-mediated cytotoxicity test was performed as follows. Briefly, ^{51}Cr labeled target cells were washed 3 times in MEM and incubated in 0.25 ml MEM + 10% foetal calf serum with effector lymphoid cells, at the MEM + 10% cell ratio 100: 1, for 6 h at 37 °C, in an atmosphere of 5% CO_2 in air. Each test was performed with 6 replicates. ^{51}Cr release was measured in the supernatants, and the percentage of cytotoxic activity was calculated according to the formula:

$$\%\ ^{51}Cr\ \text{release} = \frac{\text{test release-background release}}{\text{maximal release-background release}} \times 100.$$

Background release was determined by incubating target cells with unlabeled tumor cells in place of effector cells. This background release was 7-15% of the total label. Maximal release was calculated by treating the target cells with distilled water overnight (75-86% of the total label).

Results and discussion. Spontaneous cytotoxicity of different lymphoid cell populations from CFW/L1 mice against EAC cells in vitro is presented in the table.

In our experiments, in accordance with other reports[12,16] spleen cells displayed the highest natural cell-mediated cytotoxicity. The lowest, but significant cytotoxicity (in 2 experiments) was mediated by thymus cells. Thymus cells are known to have a low[11] or insignificant[12,16,17] natural cell-mediated cytotoxicity. Peyer's patch cells exerted a natural cell-mediated cytotoxicity against EAC cells of the same magnitude as that exerted by lymph node cells. The immunological role of Peyer's patches is not fully elucidated. Since the natural killer cells may play a role in host resistance against cancer, their well-marked presence in Peyer's patches might suggest that one of the functions of Peyer's patches is to participate in immunological surveillance against cancer of the gastrointestinal tract.

Spontaneous cytotoxicity of cells from different lymphoid organs of CFW/L1 mice against EAC cells in vitro

Cell source	Mean cytotoxicity ± SE (%) in		
	Exper. I	Exper. II	Exper. III
Spleen	11.41 ± 1.08	5.41 ± 0.57	17.08 ± 1.16
Peripheral lymph nodes	7.32 ± 2.76	2.08 ± 0.82	6.55 ± 1.13
Mesenteric lymph nodes	5.54 ± 0.81	3.18 ± 0.86	3.99 ± 0.89
Thymus	2.67 ± 0.57	1.32 ± 0.92	4.42 ± 1.55
Peyer's patches	5.86 ± 0.99	3.98 ± 0.91	10.31 ± 2.32

On the basis of the variance analysis (model of randomized blocks) the differences between the means of cytotoxic activity (CA) of the cells investigated were found at the significance level of $p < 0.05$. To check the significance of differences between individual groups of cells, the orthogonal contrasts method was additionally used. All computations were made on the transformed values of CA (arcsin $\sqrt{0.01\ CA}$) as their frequency distribution was not normal.

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