Lymphology 13 (1980) 40–43

T-Lymphocytes in Venous Blood and Lymph from the Thoracic Duct and Right Lymphatic Duct of Normal Dogs

H.S. Kaufman, M.D., S.E. Leeds, M.D., H.N. Uhley, M.D., with the technical assistance of L.B. Teleszky and V. Villaluna

Department of Medicine and the Experimental Surgery Laboratory, Mount Zion Hospital and Medical Center, San Francisco, California 94115, USA

Summary

The WBC and per cent polymorphonuclear cells in blood increased significantly during the approximately 3 hours of the experiments. The number of lymphocytes did not change significantly.

Corresponding cellular components in RD and TD lymph were not significantly different.

The per cent of T-rosettes in FVB was more than three times that in RD and TD lymph (33/10). T-rosettes were predominantly formed by medium lymphocytes (68–82%) in both blood and lymph.

The cellular components of the lymphatic system in the canine is composed of several functional types. Two major cell lines are: B-lymphocytes, initially found in the bursa of Fabricius of fowls, and T-lymphocytes which are thymus dependent. Antibodies (immunoglobulins) are produced by B-lymphocytes which mature into comitted plasma cells. T-lymphocytes are associated with cell mediated immunity. T-cells also control some B-cell activity.

B-cells may be identified by cell surface immunoglobulin when exposed to fluorescein labeled specific anti-serum. Canine T-cells are identified by the spontaneous binding of human red blood cells to the cell surface to form rosettes (1, 2).

We have previously reported comparative studies of the morphologic types of lymphocytes in peripheral blood and lymph from the right duct and thoracic duct of dogs (3). It is the purpose of this report to compare the incidence of T-cells in peripheral blood to that in lymph from the right lymphatic duct (RD) and thoracic duct (TD) of dogs.

Methods

Healthy mongrel dogs weighing 18–22 kg were anesthetized by the intravenous injection of 29 mg/kg body weight of pentobarbital sodium. Respirations were maintained by an endotracheal tube connected to a Harvard respirator. Sky Blue 4%, or Evans Blue Dye (T 1824) 0.5%, was instilled into the right main stem bronchus to aid in visualization of the right lymphatic duct and its tributaries. Through a right neck incision, a PE 160 catheter was inserted into an isolated segment of the external jugular vein into which the multiple channels of the right duct enter (4, 5). A PE 160 catheter was also inserted into the thoracic duct through a left neck incision. Both cannulations were performed without opening the thorax. Heparinized lymph samples were collected from the right duct (RD) and thoracic duct (TD). Heparinized femoral venous blood (FVB) samples were collected immediately after the induction of anesthesia, after cannulation of the RD and TD, and after a one hour collection of lymph. Thus the three FVB samples were collected over a period of approximately 3 hours.

The number of T-lymphocytes was determined by the technique of *Bowles* et al. (1), modified according to the micro-rosette test described by *Moyer* et al. (6).

Supported by the National Institutes of Health Grant # 3180, and donations from Milton Weingarten and Major and Mrs. M. Goldsmith.

0024–7766/80 1300–0040 $ 02.00

For size identification purposes, only T-cells which had an identifiable lymphocyte in the same field were included. Excluded cells were listed as unidentified.

The paired t test was used for statistical analysis of the data.

Results (Tables 1 and 2, mean values, 7 experiments)

A. *WBC in femoral venous blood (FVB).* WBC's in FVB increased significantly ($P < 0.05$) during the approximately 3 hours of experiments. The per cent of polymorphonuclear cells (PMN's) rose significantly ($P < 0.01$) and the small lymphocytes decreased significantly ($P < 0.05$). There was no significant change in the per cent medium and large lymphocytes. The per cent of eosinophils decreased significantly ($P < 0.01$). The absolute number of lymphocytes did not change significantly (3,638–3,579).

B. *WBC in lymph from RD and TD.* Comparison of corresponding cellular components in RD and TD lymph did not reveal significant differences.

C. *T-cells in femoral vein blood.* There were 33% T-cell rosettes in femoral vein blood of normal dogs. There was little change after 3 hours of anesthesia. Eighty per cent of the T-cell rosettes were formed around medium lymphocytes, 0.8% were formed by small and 2.4% by large lymphocytes.

D. *T-cells in lymph from RD and TD.* In RD lymph there were 8.9% and in TD lymph there were 10.5% T-cells; 68% of the T-cell rosettes in RD lymph were formed around medium lymphocytes, 8.4% around small and 5.2% around large lymphocytes. In TD lymph there were 82% T-cell rosettes formed around medium lymphocytes, 0.5% around small and 0.5% around large lymphocytes.

Discussion

In the canine, rosettes are spontaneously formed by lymphocytes when incubated with human red blood cells (HRBC) (1, 2). The number of rosettes which are formed is variable, and may depend on the temperature and duration of incubation, presence of serum, origin of the red blood cells, trauma to cells, etc. (7, 8).

Beall et al. (2) reported a mean of 21.7 ± 2% rosettes in the peripheral venous blood of 12 normal dogs. They state, "the number of rosette-forming cells found in the blood of normal dogs varied considerably and this same variability was noted when a single dog was examined serially. The rosettes were found only around mononuclear cells. On stained slides, polymorphonuclear cells were readily recognized and never formed rosettes".

Bowles et al. (1) found a mean of 39.8 ± 7.6% (range 23–55) E-rosettes in the peripheral venous blood of 14 normal dogs at optimum incubation of 22 °C for 30 minutes. At less than optimum temperatures and durations of incubation, lower values from 18.3–30.4% were obtained. They state, "the percentage of lymphocytes which formed E-rosettes was not significantly different ($P < 0.05$) whether the lymphocytes were incubated in MEM, Hank's balanced salt solution, PBS or vernol buffer. The addition of normal canine or fetal calf serum at 5% or 33% did not markedly alter the percentage of lymphocytes which formed E-rosettes with human RBC".

Our results fall between those of the above authors (Table 2). We incubated for 15 minutes at 37 °C and let the mixture stand overnight. *Beall* et al. (2) stored the cells at 4 °C and used both a counting chamber and dry stained smears, while *Bowles* et al. (1) found that incubation at 22 °C for 30 minutes resulted in the highest yield of T-lymphocytes.

Anesthesia for approximately 3 hours did not significantly affect the level of T-cells in peripheral blood although the number of WBC's increased significantly due to the increase in PMN's.

Certain species differences are noted when human and canine T-cells are compared. In man, when sheep RBC's are incubated with lymphocytes, rosettes are predominantly formed around small lymphocytes, whereas in the canine the medium lymphocyte appears to be the cell which forms rosettes with human RBC's.

Table 1 White blood cells and differential counts in FVB, RD and TD lymph of normal dogs (n = 7)

	Femoral vein blood (FVB)				
	Immediately after induction of anesthesia	After cannulation of RD and TD	After collection of lymph	Right duct lymph	Thoracic duct lymph
Total number of WBC	8,343 ± 1,800	11,421 ± 5,600	15,493 ± 8,900	5,707 ± 4,176	8,443 ± 3,789
		$P < 0.05$			
PMN's %	51.7 ± 15.4	66.9 ± 11.8	75.7 ± 11.2	6.3 ± 6.8	0.5 ± 1.1
		$P < 0.01$			
		$P < 0.01$			
			$P < 0.05$		
Eosinophils %	4.0 ± 4.3	3.3 ± 2.4	0.4 ± 0.8	0	0
			$P < 0.01$		
Small lymphocytes %	26.9 ± 22.6	13.4 ± 9.5	10.3 ± 8.6	68.6 ± 20.9	88.6 ± 9.7
		$P < 0.05$			
Medium lymphocytes %	15.1 ± 11.3	15.8 ± 8.9	12.7 ± 15.0	19.1 ± 15.0	9.8 ± 7.8
Large lymphocytes %	1.6 ± 2.9	0.4 ± 0.5	0.1 ± 0.4	4.0 ± 5.2	1.0 ± 2.6
Total number of lymphocytes	3,638 ± 1,026	3,379 ± 719	3,579 ± 914	5,233 ± 781	8,391 ± 565

Table 2 Percentage of T-lymphocytes in FVB, RD and TD lymph of normal dogs (n = 7)

	Femoral vein blood (FVB)			Right duct lymph	Thoracic duct lymph
	Immediately after induction of anesthesia	After cannulation of RD and TD	After collection of lymph		
% Total number T-lymphocytes*	33.0 ± 16.2	34.6 ± 11.8	33.2 ± 12.0	8.9 ± 5.6	10.5 ± 12.9
	↑	↑	P < 0.01	↑	
	↑			P < 0.01	↑
Small lymphocytes %	0.8 ± 2.0	1.0 ± 3.2	0.4 ± 1.6	8.4 ± 19.8	0.5 ± 1.2
Medium lymphocytes %	80.0 ± 10.8	74.6 ± 8.9	78.2 ± 6.5	68.4 ± 32.6	81.6 ± 23.2
Large lymphocytes %	2.4 ± 3.7	0.9 ± 2.0	2.8 ± 6.6	5.2 ± 15.0	0.5 ± 1.8
	↑ P < 0.05	↑			
Unidentified %	16.8 ± 9.0	23.4 ± 8.0	18.6 ± 7.8	18.0	17

*Total number of lymphocytes in Table 1

It has been stated that in man there are 65–85% T-cells in blood and 90–95% in TD lymph (9). In the canine we found 33 ± 16.2% in blood and 10.5 ± 12.9% in TD lymph. The reason for the difference in T-cell content of blood and lymph is not apparent at this time.

References

1 *Bowles, C.A., G.S. White, D. Lucas:* Rosette formation by canine peripheral blood lymphocytes. J. Immunol. 114 (1975) 399–402

2 *Beall, G.N., J.R. Benfield, S.R. Kruger, P.E. Byfield:* Canine lymphocytes that form rosettes with human red blood cells. J. Surg. Res. 17 (1974) 330–337

3 *Leeds, S.E., H.N. Uhley, C.M. Basch, E.H. Rosenbaum, J.M. Yoffey:* Comparative studies of lymph and lymphocytes of the thoracic duct and right lymphatic duct. I. Normal dogs. Lymphology 4 (1971) 53–57

4 *Leeds, S.E., H.N. Uhley, J.J. Sampson, M. Friedman:* A new method for measurement of lymph flow from the right duct in the dog. Am. J. Surg. 98 (1959) 211–216

5 *Uhley, H.N., S.E. Leeds, J.J. Sampson, M. Friedman:* Right duct lymph flow in dogs measured by a new method. Dis Chest 37 (1960) 532–534

6 *Moyer, R.P., R.J. Dockhorn:* A micro-rosette test for newborns. Ann. Allergy 35 (1975) 271–273

7 *Steel, C.M., J. Evans, M.A. Smith:* The sheep cell rosette test on human lymphocytes: An analysis of some variable factors in the technique. Brit. J. Haemat. 28 (1974) 245–251

8 *Heier, H.E.:* The influence of mechanical force on the rosette test for human T-lymphocytes. Scand. J. Immunol. 3 (1974) 677–681

9 *Barrett, J.T.:* Textbook of Immunology. Third edition. The C. V. Mosby Co., St. Louis, 1978

Sanford E. Leeds, M.D., P. O. Box 7921, Mount Zion Hospital and Medical Center, San Francisco, CA 94120, USA