PERIPHERAL BLOOD LYMPHOCYTE ADHERENCE TO MYELINATED TISSUE IN SQUALUS ACANTHIAS AND RAJA ERINACEA

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Mammalian lymphocytes have the capacity to adhere to myelinated tissue of central nervous system (CNS) origin (1,2). Lymphocyte adherence is restricted to white matter. Little or no adherence is seen to gray matter. The predominant human lymphocyte which adheres to myelinated tisue is the T-lymphocyte (2). Lymphocyte adherence to myelin has also been shown to be increased in patients with the chronic demyelinating disease, Multiple Sclerosis (MS) (2).

In preliminary studies in which we have investigated the specificity of human lymphocyte adherence to myelin, our results indicate that adherence is dependent on the availability of a lipoprotein, most likely proteolipid protein in the CNS myelin. Very little adherence is seen to peripheral nervous system myelin from vertebrate origins. This may be due to the lack of proteolipid protein in peripheral myelin in higher vertebrates (3). It has been shown that myelin from higher invertebrates and some lower vertebrates resembles peripheral myelin, or at least shares components (4,5). It is only with higher vertebrates that a differentiation into CNS and PNS myelin occurs (3,4) and this appears to be due to the presence of the proteolipid protein produced by CNS oligodendrocytes, and the P_o protein produced by Schwann cells of the peripheral nervous system. It was of interest therefore to determine whether a similiar lymphocyte adherence mechanism existed in lower vertebrates and/or higher invertebrates, and whether lymphocytes of human origin could adhere to myelin from the skate (Raja erinacea) and from the dogfish (Squalus acanthias).

Samples of myelinated tissue from the cerebellum of the skate and dogfish were cut coronally (0.8 microns) and frozen at -18° C. Sections were fixed in 3% glutaraldehyde and treated with lysine. Human peripheral blood mononuclear cells obtained by venipuncture and isolated by centrifugation on Ficoll-Hypaque gradients (6) were allowed to adhere for 30 minutes at 8° C. or room temperature (2). Lymphocytes were gently rotated over fixed sections at 40 rpm. Following adherence, nonadhered lymphocytes were removed by washing slides 4 X in cold phosphate buffered saline (PBS) pH 7.2. Results shown in Table 1 indicate that human lymphocytes selectively adhere to myelinated tissue from the dogfish but not from the skate. Little or no adherence was seen to gray matter from either species. Adherence observed to dogfish myelin is approximately half that normally observed to human myelin.

Preliminary experiments in which lymphocytes were allowed to adhere at room temperature and at 8° C. indicated that human lymphocyte adherence to elasmobranch myelin is maximum at 8° C. Temperature had no effect on adherence to nonmyelinated tissue (data not shown).

Experiments were also performed using dogfish peripheral blood lymphocytes obtained from the caudal vein. Samples were drawn into syringes containing heparin (100 U/ml). Blood was layered on

Ficoll-Hypaque gradients (LSM, Bionetics) at a 2:1 [LSM:NaCl(0.9%)] ratio. Gradients were spun at 400 x g for 30 min. The successful separation of dogfish peripheral blood mononuclear cells varied with the concentration of heparin, the sex of the dogfish, and perhaps the age. Cells were washed 4 X and resuspended at 1 x 10^7 cells/ml in RPMI 1640 supplemented with 0.35 M urea. A similiar technique was found to be successful for separating nurse shark lymphocytes (7). Hyder and colleagues (7) have reported no sex variation in the successful separation of nurse shark peripheral blood lymphocytes.

Dogfish lymphocytes were allowed to adhere to myelinated tissue of both skate and dogfish origin. No adherence was seen to either skate white or gray matter (Table 2). Adherence was seen only to dogfish white matter. When the concentration of lymphocytes and the temperature of adherence was varied, the results in Table 3 were obtained. When lymphocytes were allowed to adhere at concentrations of 1 x 10^7 cells/ml, no significant differences were seen between adherence at room temperature and 8° C. When concentrations of 3 x 10^7 cells/ml were used, adherence at room temperature increased, but no change was seen in adherence at 8° C. When concentrations of 5 x 10^7 cells/ml were used, adherence at both room temperature and 8° C. was significantly ($p < 0.01$) increased. The increases were greater at room temperature. An increase in adherence was also seen to gray matter, suggesting that at least part of the concentration-dependent change was due to nonspecific adherence (data not shown)

It is not known what role lymphocyte adherence to myelin may play in vertebrate neuroimmunology. The present results support our previous conclusion that human lymphocyte adherence is specific for proteolipid protein. Shark myelin has been shown to have proteolipid protein-like components (4,5). That human lymphocytes adhered only to dogfish and not to skate myelin may be potentially important. Dogfish myelin contains proteolipid proteins which have characteristics similar to peripheral nervous system P_o proteins (4,5). Our results indicate that dogfish also appears to have determinants recognized by human lymphocytes. The biochemical nature of skate myelin is not well characterized. That human lymphocytes can not adhere to skate myelin suggests that it does not possess the necessary receptors required for adherence. By reference this suggests that skate myelin either lacks proteolipid protein or lacks the proteolipid protein-P_o protein determinants seen in dogfish myelin.

Experiments in which dogfish lymphocytes were shown to selectively adhere to dogfish myelin suggests that a system similar to human lymphocyte adherence to myelin exists in elasmobranchs. Preliminary experiments with skate lymphocytes indicates that these cells are not able to adhere in significant numbers to either skate or dogfish myelin. It is possible that the appearance of peripheral blood leukocytes which recognize myelin in the dogfish coincides with differentiation of PNS and CNS myelin. Further investigation of lymphocyte adherence in the dogfish may yield exciting new insights as to the role this system plays in neuroimmunology and perhaps its relationship to tissue injury in multiple sclerosis.

References

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TABLE 1: HUMAN LYMPHOCYTE ADHERENCE TO MYELINATED TISSUE FROM THE DOGFISH AND SKATE.

	# LYMPHOCYTES/10^5 uM^2 *	
	SKATE	DOGFISH
WHITE MATTER	1.2 +/- 0.2	21.3 +/- 3 **
GRAY MATTER	0.8 +/- 0.04	0.68 +/- 0.01
n***	7	6

* Mean +/- S.E.

** Statistically significant ($p<0.01$)

***Number of individual experiments. Each experiment involved different samples of human cells.

TABLE 2: DOGFISH LYMPHOCYTE ADHERENCE TO MYELINATED TISSUE FROM DOGFISH AND SKATE.

# LYMPHOCYTES/10^5 uM^2*	
DOGFISH	SKATE
4.2 +/- 1	--

* Mean +/- S.E.

** n = 4 experiments

TABLE 3: DOGFISH LYMPHOCYTE ADHERENCE: EFFECT OF TEMPERATURE AND CONCENTRATION.

	# LYMPHOCYTES/10^5 uM^2*	
	30° C.	8° C.
1 x 10^7 cells/ml	4.7 +/- 0.5 (3)	5.5 +/- 1 (3)
3 x 10^7 cells/ml	7.6 +/- 1.0 (3)	4.3 +/- 0.5 (3)
5 x 10^7 cells/ml	21.2 +/- 2 (3)	11 +/- 2 (3)

* Mean +/- S.D.

( ) = Number of experiments.