Direct contacts between nerve endings and lymphoid cells in the jugular body of *Rana pipiens*[1]

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Summary. The ultrastructure of contacts between nerve terminals and lymphoid cells in a lymphoid myeloid organ, the jugular body, of *Rana pipiens*, has been analyzed. The results are discussed emphasizing their importance for functional relationships between the neuroendocrine and lymphoid systems.

In addition to intrinsic regulatory mechanisms of immune reactions, alternative, purely physiological means of effecting immunoregulation have been tentatively proposed[5]. Experimental data show that the immune system is under external regulation by the neuroendocrine system 6,7. This evidence derives from experiments showing that hormones and neurotransmitters affect the lymphoid system's functions and, reciprocally, that immune responses cause changes in blood hormone levels[8] and in electrical activity of neurons in certain hypothalamic areas[9]. Other than this physiological evidence, there has been little interest in morphologic aspects of immuno-neuroendocrine relationships, especially in the existence of nerves within lymphoid organs as they may relate to lymphocytes. In the present work, we have analyzed, ultrastructurally, nerve endings within the stroma of a lymphomyeloid organ, the jugular body in leopard frogs *(Rana pipiens)* as morphologic evidence of proposed intimate immuno-neuroendocrine relationships. This organ was rediscovered and described along with others in *Rana catesbeiana* during the late 1960's[10].

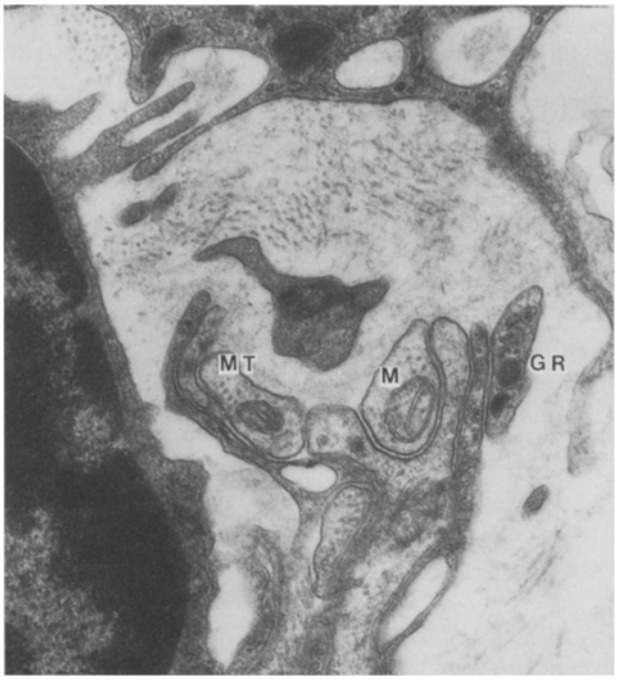
Materials and methods. Adult leopard frogs, *Rana pipiens*, 3–3½ inches body length, were obtained from Mogul-Ed (Oshkosh, Wisconsin) and maintained at 20±1 °C in semi-aquatic conditions. They were fed mealworms 2–3 times weekly and used within 4 weeks. Frogs were double-pithed and both jugular bodies removed aseptically. The jugular bodies were fixed in toto by immersion in 2.5% glutaraldehyde buffered to pH 7.3 with Millonig liquid, post-fixed in 1% osmic tetroxide in the same buffer, dehydrated in acetone and embedded in Araldite. Ultrathin sections were obtained with a Reichert OM-U3 ultratome, double-stained with lead citrate and uranyl acetate and examined with a Jeol 100-B electron microscope. Semi-thin sections approximately 1–2 µm thick were cut for tissues processed for electron microscopy. These sections were stained with an alkaline solution of toluidine blue.

Results. We confirmed that the jugular body of *Rana pipiens* was surrounded by a capsule of connective tissue and squamous epithelium. The internal parenchyma consists of cell cords arranged in a sinusoidal blood vessel network. The cell cords were formed by a stroma of irregular reticular cells and by free cells, which consist of small and medium lymphocytes, lymphoblasts and developing and mature plasma cells. Moreover, free macrophages, neutrophils and eosinophils were also present. Innervation was principally by means of vasomotor nerves with non-myelinated nerve endings near the basement membranes of sinusoidal endothelia. In addition, sometimes nerve endings appeared in proximity to free cells or fixed reticular elements.

An unmyelinated nerve ending in contact with a small lymphocyte is shown in the figure. This ending showed several distinct axoplasms with mitochondria and microtubules. In some of them, there were round granules containing an electron-dense core and a light peripheral halo. At other times, the interactions between nerve endings and cells in the jugular body appeared closer. Nerves have actually been observed directly on cells and appear to be surrounded by processes from them. Some of the axons seemed to make contact with the cell membrane. although synaptic contacts never appeared. The morphology of these nerves was similar to that described in the figure, although the amount of electron-dense granules in the axoplasm of axons making direct contact with the reticular membrane was remarkable.

Discussion. The existence of nerves within lymphoid organs and their relationships with lymphocytes has received little or no attention. Some authors have reported unmyelinated nerves adjacent to reticular cells and lymphocytes in mammalian splenic white pulp[11–13] and bone marrow[14–17]. While the physiologic significance of the relationship of these nerves to reticular cells is not clear, some reports[18–21] indicated that β and α adrenergic receptors may influence or modulate the cell cycle of hemopoietic stem cells or even lymphocytes. Our morphologic data suggest that the electron dense vesicles described in the nerve terminations may contain biogenic amines, perhaps nor-adrenalin.

Recently, Hodgson et al.[22] showed that adrenaline stimulated α or β adrenoreceptors of cells from 4 anuran species, producing an α effect (a reduction in rosette formation) in the presence of β antagonists and a β effect (an increase) in the presence of α antagonists. This same stimulation resulted in a reduction in rosette formation by cells from 5 urodeles. In addition, Besedovsky et al.[5] have suggested that changes in local concentrations of neruotransmitters



Nerve ending in close relationship with the membrane of a small lymphocyte. Note its unmyelinated nature and the presence in the axoplasms of microtubules (MT), mitchondria (M), and granules with an electron-dense core (GR). × 7800.

act as regulatory signals capable of modulating the immune response. Therefore, it is tempting to speculate that the contacts between nerve endings and cells of the immune system: 1) might influence or modulate the cell cycle of lymphoid cells or their capacities for reacting in immune responses or, 2) modify the microenvironments of lymphoid organs through the modulations of reticular cells.

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Effect of cyclophosphamide on development of reticulum cell sarcoma in SJL/J mice[1]

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Summary. Studies to determine the effect of cyclophosphamide (CY) on the development of reticulum cell sarcoma (RCS) in SJL/J mice indicated a dependence on the duration of the test period. Age also appeared a factor of importance. Thus, a comparison of tumor incidences at 52 weeks of age showed maximal inhibition when CY was administered at 40 weeks, minimal inhibition when the drug was given at 30 weeks, and intermediate inhibition when treatment was initiated at 10 and 20 weeks. Consistent with these findings, long-term treatment of 40-week-old SJL/J mice with low doses of CY resulted in an increase in the mean survival time and in a reduction in the incidence of RCS.

The SJL/J strain of mice was derived from noninbred Swiss Webster stock by brother × sister matings for 30 generations. Initial reports emphasized the susceptibility to development of type B reticulum cell sarcoma (RCS) in high incidence, and the shared features of this spontaneous disease of mice and Hodgkin's disease of man[2,3]. Thereafter, other characteristics of the strain were described such as low erythrocyte counts (males), a high incidence of spontaneous amyloidosis (old females), resistance to tolerance induction, a high susceptibility to induction of delayed hypersensitivity, paraproteinemia and antinuclear antibodies[4–6].

Consistent with the many immunologic manifestations, most attempts to inhibit the development of SJL/J neoplasms have had their basis in a modification of immune functions. These include treatment with antilymphocytic serum or corticosteroids[7,8], as well as thymectomy or splenectomy and the transplantation of allogeneic bone marrow[9,10].

The purpose of this study was to determine the influence of an immunosuppressive drug, cyclophosphamide (CY), on neoplasia in SJL/J mice. A brief description of preliminary findings has been reported previously[11].

Materials and methods. Animals. Female SJL/J mice were obtained from the closed breeding colony maintained in the Animal Services Center, University of Alabama in Birmingham. The mice were caged in groups of 3 or 4, and provided with food and water ad libitum.

Test procedure. CY was prepared at a concentration of 15 mg/ml in cold 0.85% saline and administered by i.p. injection (1.5 mg/mouse/week). The age of the mice varied among the groups from 10 to 40 weeks and, in most tests, the period of treatment was either 6 or 12 weeks in length. Groups of control animals received weekly an i.p. injection of 0.1 ml of 0.85% saline, or they were not injected.

Tumor incidence. Experimental and control mice were killed at 40, 52 and 60 weeks of age, or they were held until moribund. Thereafter, the animals were necropsied for evidence of neoplasia. The findings of enlargement of Peyer's patches, the mesenteric lymph node complex, other lymph nodes and/or the spleen were accepted as indicative of RCS. In those cases in which the gross observations seemed questionable, sections of appropriate organs were prepared by routine histologic methods and examined microscopically to allow for a definitive diagnosis. Differences in tumor incidence between experimental and control mice were evaluated for statistical significance by the contingency test.

Results and discussion. Experiment 1. Two groups of 10-week-old female SJL/J mice were given a series of 6 or 12 weekly injections of CY. For controls, 15 animals received