CHAPTER II: Hypothesis and Research Objectives

The adult thymus was long thought to be vestigial. However, the discovery that neonatal thymectomy in mice (Miller 1962) or congenital athymia in this species (the nude mouse model discovered by Flanagan in 1966) led to a profound immunodeficiency as well as to endocrine alterations (Biachi, Pierpaoli et al. 1971; Pierpaoli and Sorkin 1972), revealed the crucial role of this organ in immune and endocrine maturation. This was supported by the observation that those immunological alterations could be reversed by neonatal transplantation of a neonatal thymus. In the early '60s it became apparent that certain thymic functions could even be attributed to the action of thymic humoral factors or hormones, as indicated by the fact that thymus transplants that were placed into cell-impenetrable millipore chambers could induce lymphoid tissue differentiation and immunological functions when grafted to neonatally thymectomized mice (Levey, Trainin et al. 1963; Osoba and Miller 1963). Neonatally thymectomized female mice showed the same phenomenon when they eventually got pregnant, suggesting that the thymus of the developing fetus would be able to exert any kind of long-distance-influence on the mother's tissues.

A few years after these initial discoveries, the presence of the thymus gland during early life appeared to be essential not only for normal development of the immune system, but also for the proper maturation of the hypothalamic-pituitary-ovarian axis (Besedovsky and Sorkin 1974; Lintern-Moore and Pantelouris 1975a; Lintern-Moore and Pantelouris 1975b; Rebar, Morandini et al. 1981). Congenitally athymic mice showed severe deficiencies in their reproductive function such as delayed times of vaginal opening and first ovulation, reduced fertility, and increased follicular atresia with resulting premature ovarian failure. Again, the same abnormalities were found in normal female mice after neonatal thymectomy (Michael, Taguchi et al. 1980; Nishizuka and Sakakura 1971). Interestingly, a reduced basal secretion of gonadotropins was found in nude mice by Rebar et al. (Rebar, Morandini et al. 1981; Rebar, Morandini et al. 1982) and Goya et al. (Goya, Console et al. 2001). Furthermore, the hypothalamo-pituitary-adrenal axis appeared to be hypofunctional in nude mice. Thus, it has been reported that stress stimuli acting at hypothalamic level (i.e. ether vapor inhalation or insulin administration) induce a lower ACTH and corticosterone increase in nude than in normal mice (Daneva, Spinedi et al. 1995).

Therefore, it was thought that congenital athymia brings about a functional panhypopituitarism in the mouse. This hypothesis was consistent with more recent reports showing that thymulin was able to stimulate the release of prolactin and thyrotropin (Brown, Sosa et al.

1998), growth hormone (Brown, Sosa et al. 1999), adrenocorticotropin (Hadley, Rantle et al. 1997), as well as LH and FSH (Brown, Sosa et al. 2000) from rat pituitary cells *in vitro*.

From then on, the key role of the thymic hormone thymulin as a mediator of thymus-neuroendocrine communication began to be recognized. Based on this evidence, a general facilitatory action of thymulin on anterior pituitary (AP) hormone secretion was proposed (Goya, Brown et al. 1999).

In the present work we wanted to test the hypothesis that thymulin is crucial during early life for the development of a proper neuroendocrine balance. To achieve this objective, experiments involving specific immunoneutralization of this hormone during early life were implemented. Furthermore, we sought to detect changes in other developmental aspects of the animal, such as the animal's weight gain, alteration of the animal's external appearance, signs of delayed puberty, deficiencies in their reproductive function, as well as alterations in the thymus' cell content. Unfortunately, the unavailability of relatively large amounts of anti-thymulin antibodies had hitherto prevented the performance of *in vivo* anti-thymulin quenching studies. With the introduction of avian antibodies, this need was met. Furthermore, the generation of avian antibodies against a highly preserved peptide such as thymulin promised to give rise to a highly specific pool of antibodies. In order to verify those expected advantages of IgY for our purposes, it was planned for an ELISA for titration of anti-thymulin specific mammalian antibodies and another one for avian antibodies to be set up. Other well established methods were used to characterize and compare the antibodies generated.

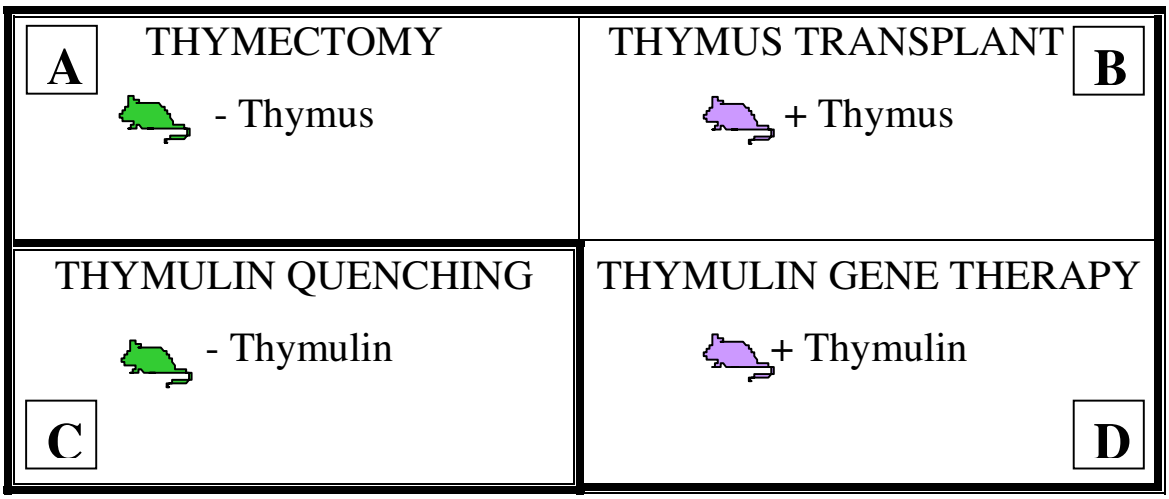

Figure 8: Four Animal Models - Models **A** and **C** involve deletion of thymus/thymulin from normal subjects (green), whereas models **B** and **D** are replacement therapies in nude or neonatally thymectomized animals (blue).