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Reciprocal interdependence between pineal gland and avian immune system

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Abstract

The immune system of mammals and birds exhibits the same basic anatomical and functional organization, including dichotomy into the cellular and humoral immune response. Specificities of avian immune system may be, however, very useful for understanding numerous phylogenetic and evolutionary mysteries. Similarities and differences between mammals and birds in terms of several pineal gland functions are well known, and they seem to include the immunomodulatory activity of melatonin (MEL) as well. Embryonic pinealectomy of the chicken demonstrated functional interrelationships between the development of the pineal gland, immune system and/or neuroendocrine network, and embryonic bursectomy influenced the diurnal rhythm of the pineal gland function and abolished the effect of immunization on serum MEL level. Also immunization with a thymo-dependent antigen (SRBC) evoked some changes in the chicken nocturnal pineal NAT activity. We have found that the pineal gland and MEL control the diurnal rhythm of immunity in the chicken, but we were not able to demonstrate any immunostimulatory and anti-glucocorticoid MEL effects, regardless of the chicken's age, sex, season, and hormone dose used. The existence of functional connections between the pineal gland and the immune system in chickens was, however, confirmed in other experimental approaches. Specific and reversible binding of 2-[^{125}I]iodoMEL to the membrane preparations from lymphoid glands was demonstrated in several avian species. In vitro MEL diminished lymphocyte proliferation stimulated by the common T-cell mitogens, while alone failed to influence the blast formation. Reciprocal functional connections between the avian immune system and the pineal gland seem to be well documented, but the mechanism(s) involved have to be elucidated.

NEL REVIEW

Particularity of the avian immune system

In 1956 a milestone in the development of modern immunology was the discovery by Bruce Glick, from the Ohio State University, that the avian cloacal gland, bursa of Fabricius, is a lymph gland containing lymphoid follicles and epithelial cells, and in both growth and histological structure it resembles the thymus and therefore may be nicknamed a "cloacal thymus" [1].

Chicken bursa of Fabricius is a blind sac connected to the dorsal wall of the cloaca at its junction with the large intestine. Bursal mucosa exhibits 11–13 longitudinal folds or plicae protruding into the bursal lumen and lined with a pseudostratified epithelium. Its lamina propria shows about 8,000 to 12,000 lymphoid follicles, organized in a central medulla and a peripheral cortex, which seem to be functionally independent compartments [2].

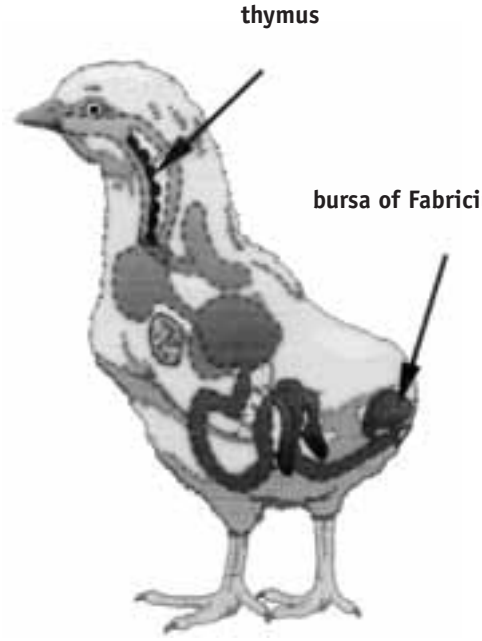

Fig. 1 Localization of the primary lymphoid glands in chicken.

Bruce Glick was also the first who demonstrated not only the existence of interrelationships between the weight and development of the bursa and testes, but he also found that the strains of chicken with heavier, therefore better developed bursa (e.g. White Leghorn vs. Rod Island Red), were more resistant to bacterial infections (e.g. *Salmonella pullorum)* [1]. Thereafter, it was demonstrated that the surgical bursectomy interferes with the development of antibody synthesis and exerts a suppressive effect on normal ontogeny of immune response [3]. These already historical considerations have created a background for understanding the vertebrate humoral immune response development and function. Avian bursa of Fabricius emerged from that as a primary lymphoid gland responsible for the normal development of antibody synthesis; therefore the lymphocytes able to produce them were named the B-cells in all vertebrate species while the bursa of Fabricius is a lymphoid gland existing only in the avian species. As the bursal follicles create a special environment where the B-cell precursors undergo maturation and start to express the surface immunoglobulin markers, several classical ideas on the immune system development and functions emerged from the study of birds, and in particular

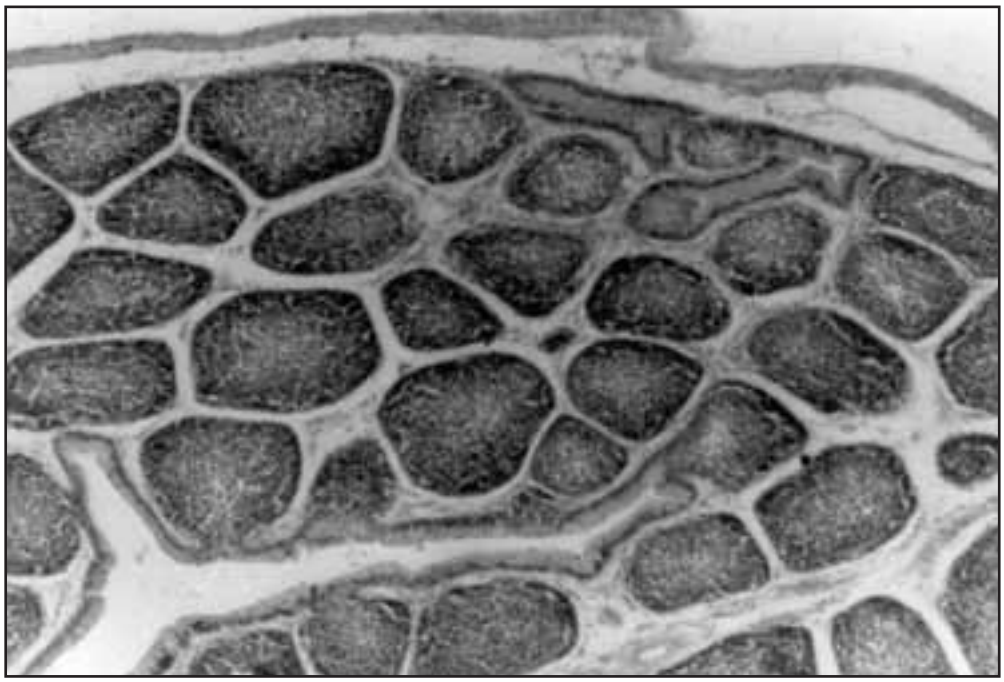

Fig. 2. Histology of the bursa of Fabricius of a 1-week-old chicken. Staining with hematoxylin-eosin, x 500; original photo by Pawel Okulski.

of the domestic fowl. Additionally, the birds also offer many methodological advantages, e.g. anatomical separation of the two primary lymphatic primordia or extramaternal development facilitating a manipulation on the embryo. A comparative research revealed that the mammalian and avian immune systems exhibit the same basic anatomical and functional organization, i.e. fundamental dichotomy into cellular, a thymo-dependent and humoral, a bursa- or bursa-equivalent-dependent immunity. This dichotomy is observed not only in higher vertebrates (endotherms, i.e. birds and mammals) but also in lower ones (ectotherms, i.e. fish, amphibians and reptiles). While the histological structure and function of the thymus is relatively similar in both mammals and birds, the structures responsible for the humoral immune response as well as immunoglobulins secreted in both species are quite different.

Avian pineal gland

The differences between avian and mammalian species in terms of pineal gland anatomy and function are well known [4]. First, similarly as in lower vertebrates, the avian pinealocytes exhibit the direct photosensitivity lost by those in mammals. Next, information on external lighting conditions, coming from the retina, evokes the different effects in both species: in mammals noradrenaline released from postganglionic sympathetic terminals during darkness stimulates MEL synthesis in pinealocytes via β-and α1-adrenergic receptors. On the contrary, in chicken, adrenergic impulsations during the light inhibit MEL synthesis via α2-adrenergic receptors. Finally, chicken pinealocytes in culture spontaneously exhibit rhythmic synthesis and release of MEL that persist for several cycles even in constant darkness, while in mammals this is not the case [5].

Taken together, there are several reasons to examine the existence of the bi-directional relationships between the pineal gland and the immune system in birds and to compare them with those in mammals.

Effect of embryonic pinealectomy on the avian immune system and bursectomy on the pineal gland

In mammals, the effect of pinealectomy on the development of immunity is equivocal and strongly depends on species and time of ontogeny when the surgery was performed. While a surgical pinealectomy during early embryonic development of mammals is methodologically difficult, if not impossible, the avian embryo offers an excellent model for this kind of the study. In chicken embryos pinealectomized at 96 h of incubation, therefore developing without any influence of the pineal gland, Jankovic and co-workers [6] have found a retarded development and decreased cellularity of both the thymus and bursa of Fabricius, a decreased humoral immune response, measured by the PFC number in the bursa and spleen, as well as a diminishing of several parameters of cell-mediated immunity. These effects were accompanied by the significant changes in the concentration of biogenic amines (serotonin, dopamine and noradrenaline) in the spleen, brain and hypothalamus. These results clearly proved the existence of functional interrelationships between the pineal gland and the development of the immune system and/or neuroendocrine network.

On the other hand, the B-cells undergo the development and maturation in bursal microenvironment composed with the different type of epithelial cells and soluble products. One of these factors was recognized as a low-molecular bursal hormone, named bursopoietin or bursin [7], a tripeptide LYS-HIS-GLY-NH_2 that induces the development of B-cells from their avian and mammalian precursors in vitro. Recently [8, 9] it was demonstrated that early embryonic bursectomy not only diminished chicken humoral immune response but also influenced the circadian rhythm of pineal gland function (pineal NAT activity, serum MEL level) as well as the MEL response to multiple immunization with porcine thyreoglobulin (Tg, see below). Both effects were reversed by bursin injected twice into the bursectomized embryo in very low, femtomolar and lower doses, indicating again the existence of the functional connection between the chicken immune system and the pineal gland function.

Effect of immunization on the pineal gland function in chicken

In seven-week-old chicken immunized three times at 9-day intervals with porcine Tg, Youbicier, Simo et al. [9] have demonstrated an increase in the diurnal serum MEL concentration after second antigenic challenge. Our own unpublished results also indicated the effect of immunization on the chicken pineal gland function, measured by the pineal NAT activity (Skwarlo-Sonta et al., unpublished results). The effect exerted by single immunization with sheep red blood cells (SRBC) was dependent on the sex and season: in winter in both sexes a nocturnal NAT activity was negatively correlated with serum anti-SRBC antibody level, whereas a similar effect in spring was seen only in females. In males in

spring the same immunization evoked a less pronounced effect, but there was rather a positive correlation between both parameters. There are several possible explanations of this difference in reaction of the chicken pineal gland to immunization; the most important seem to be the kind of antigen used (a particulate SRBC vs. soluble Tg), number of antigenic stimulation (single vs. multiple), and parameter measured (nocturnal pineal NAT activity vs. diurnal serum MEL level). Moreover, we have no information about the sex of the chickens examined and the season in which the experiments with Tg-immunized birds were performed [9].

Effect of MEL on the chicken immune response in vivo

Our preliminary experiments on chickens have demonstrated that early postnatal pinealectomy abolished circadian rhythm in several immune parameters which was restored by prolonged treatment with very low, physiological MEL doses [10]. The effects evoked by the same MEL treatment in intact birds was quite different: it depended on parameter examined, lighting conditions and MEL dose [11, 12]. Of interest was an observation that in continuous lighting the circadian rhythms of immune parameters examined disappeared and that pinealectomy, but not the sham operation, restored its circadian rhythmicity. On the contrary, neither pinealectomy nor MEL treatment influenced the level of immune parameters in chickens, indicating that in this species, at least in experimental conditions used, MEL influenced the circadian rhythm but did not exhibit the immunostimulatory and anti-glucocorticoid activity [12, 13].

It has to be pointed out that the experiments on unoperated, MEL and/or corticosterone-treated chickens were made according to the experimental protocol in which the hormone was efficient as an immunostimulatory and anti-stress agent in mice [14–16]. Some of the MEL-treated chickens were also injected with the opioid antagonists naltrexone or naloxone to examine whether the endogenous opioid system (EOS) may be involved in the MEL effect on immunity in chickens [13], as it was found in mice [16]. Both MEL and opioid antagonists diminished spleen PFC number in chickens, whereas in mice the stimulatory effect of MEL was antagonized by naltrexone [12].

Subsequent experiments were done in the spring and winter on males and females at different ages (3–5 week-old) kept from hatching in L:D=12:12 conditions (unpublished data). In the majority of cases MEL treatment antagonized the effect of control injections with PBS, thus the effect of handling: is seen because an additional, intact control group was used. The lack of immunostimulatory effect of MEL on chicken immune parameters in vivo was observed in our experimental protocol regardless of the chicken's age, sex and season. It suggests that in chickens MEL may act as a regulator of the circadian rhythm and exert some anti-stress immunoprotection but not in the case when exogenous corticosterone was added.

The only difference between experimental protocol used to examine the effect of MEL on immunity in mice [14, 15] and chicken consisted in the age of the animals used. Actually, the animals in Maestroni's experiments were young, sexually mature female mice, whereas we used very young, 3–5-week-old female and male chickens, in which the sexual maturity starts at the age of 15–18 weeks. As the immune system is influenced also by sexual hormones [17] and the effect of MEL on several physiological parameters was demonstrated to be gender-dependent, not only the systematic distance but also the difference in sexual maturity may be among the reasons for the different MEL activity observed within the immune system of mice and chicken. However, results obtained by Rodriguez and Lea [18] in adult male ring doves indicated that pinealectomy evoked an increase of several non-specific immune parameters, suggesting that also in this avian species the pineal hormone is not an immunostimulatory agent.

MEL receptors within the avian immune system

When MEL receptors started to be discovered outside the central nervous system, Yu and co-workers [19] were the first who demonstrated their presence in avian spleens with higher density in chickens and ducks than in mice. Subsequently, specific, reversible and high affinity binding of 2-[^{125}I]iodo-MEL was described in membrane preparations from avian spleen (chicken, pigeon, quail) [20, 21], thymus (duck) and bursa of Fabricius (duck) [20, 22–25]. MEL binding sites in the avian lymphoid glands fulfill all functional criteria suggesting that they belong to the M1 class of MEL receptors proposed by Dubocovich [26]. Our own data [27] revealed in 4-week-old cockerels MEL binding by membrane preparations isolated from whole lymphoid glands, much lower than in the brain. Of the lymphoid tissues, the highest 2-[^{125}I]iodo-MEL binding was found in the bursa of Fabricius, much lower in the spleen and only traces in the thymus. Neither Kd nor density of MEL binding sites in those chickens were modified

by immunization with SRBC, which caused a significant immune response, measured by the serum anti-SRBC agglutinin level. Therefore, it was suggested that in immunocompetent birds, MEL binding by both primary (thymus and bursa of Fabricius) and secondary (spleen) lymphoid organs is unrelated to the immune system activation by a T-dependent antigen [28]. These results are in disagreement with those obtained by Poon et al. [24] in 2-week-old ducks treated for 7 days with pharmacological doses of cortisol, in which a significant reduction of Bmax was observed and attributed to a change in the immune status of cortisol-treated birds. On the other hand, Wang et al. [21] have reported a significant increase in the number of MEL binding sites on spleen membrane preparations from hydrocortisone-treated pigeons. It remains to be established whether or not MEL binding sites within the avian immune system are directly involved in the immunomodulatory activity of this hormone. It is worthwhile to stress that when membranes were prepared from isolated lymphocytes and remained tissue debris containing epithelial cells, blood vessels and, probably, some remained lymphocytes, both subfractions of respective lymphoid glands exhibited ability to bind MEL (Dziwinski et al. in preparation). These results imply the possible participation of MEL in the intratissue microenvironment formation and/or development and maturation of immunocompetent cells.

Effect of MEL on chicken lymphocytes in vitro

Simultaneously, we have started to examine the effect of MEL on chicken immune cells in vitro [29]. As expected, MEL alone added to the lymphocyte cultures in wide range of concentration did not influence the cell proliferation, measured by the ^{3}H-thymidine incorporation. In cultures stimulated with the common T-cell mitogens, the MEL addition generally diminished the cell proliferation and the effect was the best seen in the cultures of lymphocytes isolated from the youngest chickens examined (5-days-old). MEL added to the cell culture simultaneously with the mitogen or 2 h earlier exerted similar effect on lymphocyte proliferation. But, when the splenocytes were pretreated with the mitogen for 2 h, MEL addition blocked almost completely the blast formation (Markowska et al., unpublished data). As Ca^{2+} is involved in the early lymphocyte activation [30], and, on the other hand, the best known mechanism of MEL action on the rat pituitary cells stimulated by forskolin or GnRH is also based on the influence on Ca^{2+} channels and intracellular Ca^{2+} concentration [31, 32], it seems to be worthwhile to continue this line of research on the mechanism of MEL action in the avian immune system.

Concluding remarks

Taken together, results existing to date seem to suggest that in chickens, similarly as in mice, MEL may be involved in the development, maturation and function of the immune system, but the mechanism(s) operating may be different. This suggestion is in line with our recent preliminary indications, that in chickens the immune system MEL may also operate via endogenous opioids, but that the effects exerted by EOS are different in mammals and birds. First, using a RT-PCR method, we have found that in lymphoid glands obtained from MEL-treated chickens there is an expression of POMC gene (Dziwinski, unpublished data). Moreover, in chickens injected intraperitoneally with thioglycollate, we have observed that morphine addition increased and prolonged a local inflammatory reaction, in contrast with the effect observed in the same experimental protocol in mice (unpublished data). The effect of MEL depended on the phase of inflammatory process: at the moment of maximal inflammatory response it diminished the leukocyte number in peritoneal exudate, but in the descending phase of inflammation an increase caused by MEL was noted. Of interest is an observation that, given together, both agents diminished local inflammatory reaction, regardless of the effect exerted separately. These in vivo results deserve further research, in particular concerning the effect of EOS and morphine-like opiates on chicken immune parameters, to our knowledge not examined to date.

Acknowledgments

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