Development of the Innate Immune Response in Nestling Tree Swallows (Tachycineta bicolor)

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Abstract

The innate immune system provides an immediate, short term, first line of defense from pathogens; its appearance early in development in vertebrates is evidence of its critical importance. Even so, few studies have investigated the development of the immune response as juveniles transition into adults. Ultimately, the ability to respond to pathogens confers fitness benefits in terms of health, survival, and reproductive success, and it facilitates that functions such as rapid growth cannot be fully met simultaneously since energy is a limiting resource. As a result, defense mechanisms are compromised at an early age due to energy allocation to rapid growth; therefore, immunity should increase as individuals mature. I studied the development of innate immunity in nestling Tree Swallows using microbicidal assays, which were conducted in vitro to assess the ability of the immune system to kill E. coli via lysis. This research may provide insight into patterns of disease susceptibility, which in turn influence evolutionary fitness and population dynamics.

Introduction

Ecoimmunology seeks to explore and understand various immune defense strategies observed among species. The ecological study of the development of immune function in birds and other vertebrates is in its infancy [1], and age-specific variation in immune function has been understudied in regards to innate immune development in wild animals [2, 1].

The vertebrate immune system consists of two components: innate and acquired immunity [3]. Innate immunity is the nonspecific response to pathogens through complement, a cascade of proteins acting in sequence to produce biological effects concerned with inflammation and the lysis of cells, and phagocytosis, macrophages that ingest foreign particulate matter by enveloping them into the cell, occurring in the blood. Unlike acquired immunity, which develops over the lifetime of an organism from previous encounters with pathogens, innate immunity is present at birth. Innate immunity provides an immediate, short-term, first line of defense that develops throughout the juvenile stage. Because these components have a genetic basis, they are directly subjected to natural selection, thus favoring the evolution of immune competence, and observations that the components of the innate immune system appear early in development in vertebrates [2, 4], including Tree Swallows, Tachycineta bicolor [1], are evidence of its critical importance. Immune function involves life-history trade-offs influenced by differences in energy allocation to growth, maintenance and reproduction [5], all of which are energetically costly [6]; thus these three processes cannot be fully met simultaneously. Juveniles use energy for growth and maintenance, whereas adults use energy for maintenance and reproduction. Ultimately, the ability to respond to pathogens confers fitness benefits in terms of health, survival, and reproductive success [7]. Selection should favor those individuals that are able to budget their energy so as to balance the demands of growth and maintenance. This demand results in strong selection pressures on developing songbirds, which must grow rapidly to be able to leave the nest and avoid predation [8] while at the same time develop a competent innate immune response.

Most ecoimmunological studies have not focused on the development of innate immunity in immature, wild animals, but rather have used domesticated species such as chickens with precocial development [9, 10] as opposed to altricial development found in wild Tree Swallows and other passerines. Furthermore, chickens have been artificially bred for increased body growth for meat production or egg-laying, which may alter their lifespan, thus possibly altering natural, innate immune defense mechanisms [11, 12]. Additionally, in those studies that do exist on wild species, measurements were primarily taken at one specific age (e.g., 12 days after hatching), neglecting developmental stages as juveniles transition into adults.

Studies on the development of immune defense in nestling Tree Swallows may provide insight into patterns of age-related disease susceptibility, which in turn influence evolutionary fitness and population dynamics [13]. I studied the development of innate immunity in nestling Tree Swallows to

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immunity in a free-living, altricial bird, the Tree Swallow. Microbicidal assays [14] were used to study the role of innate immunity in a free-living, altricial bird species, the Tree Swallow (Tachycineta bicolor). Blood samples were collected within 3 to one end. Upon venipuncture, the area on the wing was sterilized with 70% ethanol. Venipuncture and blood collection occurred after the ethanol completely evaporated because ethanol can cause hemolysis, which can complicate immune assays [14]. Capillary tubes were sealed with clay and placed in sterilized containers for transport to the lab. Blood was transported to the laboratory usually within 60 min. of collection for the best results during immune assays [14]. The microbicidal assay produces an index of the capacity of the blood, measuring how well blood can kill microbes after incubation/number inoculated (10 µL of working culture per 100 µL of media) and then immediately plated. Negative controls in which no microbes were added were also prepared. Microbicidal activity of the blood was defined as the percent of the inoculum killed which equal 1 (visible microbes after incubation/number inoculated) [14].

Results

corticosterone, which can affect immune response, remains at or near baseline levels in the field and laboratory [15, 16]. Blood was collected into sterile heparinized capillary tubes (50-75 µL) capacity directly from the brachial vein in the wing after venipuncture using sterile lancets or needles. Prior to venipuncture, the area on the wing was sterilized with 70% ethanol. Venipuncture and blood collection occurred after the ethanol completely evaporated because ethanol can cause hemolysis, which can complicate immune assays [14]. Capillary tubes were sealed with clay and placed in sterilized containers for transport to the lab. Blood was transported to the laboratory usually within 60 min. of collection for the best results during immune assays [14]. The microbicidal assay produces an index of the capacity of the blood, measuring how well blood can kill microbes after incubation/number inoculated (10 µL of working culture per 100 µL of media) and then immediately plated. Negative controls in which no microbes were added were also prepared. Microbicidal activity of the blood was defined as the percent of the inoculum killed which equal 1 (visible microbes after incubation/number inoculated) [14].

Methods

Tree Swallows are a common, annually for- pearing species distributed throughout North America that readily accept nest boxes. The nest boxes I used were a series of clutchless of 5-6 eggs which hatch after ap- proximately 15 days of incubation display- ing. Hatchlings are altricial, and both par- ents feed the nestlings up until the time they fledg, which is about 20 days [15]. I studied Tree Swallows that nested in wooden nest boxes, which is an abundant nest site in the campus of Grand Valley State Univer- sity (GVSU) (42º57´N, 85º53´W), Ottawa County, Michigan in June and early July, 2009. Nestlings were monitored closely to de- termine clutch completion date, exact hatch date, and, therefore, exact nestling age. I obtained blood samples from 86 nest- ing sites during the period from the day the first egg in a clutch hatched) 6, and they fledg approximately 20 days after hatching [15]. I examined the innate immune system at nestling days (ND), 0-6 (the day the first egg in a clutch hatched) 6, 12, and 18, which fall in the range of these developmental stages. At ND-3, nestlings were too small to obtain good blood samples of adequate volume to perform in vitro assays, so I began drawing blood from nest- ing nestlings, which weigh 10-12 g, about half as much as adult swallows weigh [15]. At ND- 12, Tree Swallows can feed themselves, and I obtained samples from the nestlings that are nearly the 0.2 g with a Pesola spring, and flattened wing chord of the right wing to the nestlings with a ruler with a stop fixed to one end. Blood samples were collected within 3 min. of handling because the stress hormone corticosterone, which can affect immune response, remains at or near baseline levels in the field and laboratory [15, 16]. Blood was collected into sterile heparinized capillary tubes (50-75 µL) capacity directly from the brachial vein in the wing after venipuncture using sterile lancets or needles. Prior to venipuncture, the area on the wing was sterilized with 70% ethanol. Venipuncture and blood collection occurred after the ethanol completely evaporated because ethanol can cause hemolysis, which can complicate immune assays [14]. Capillary tubes were sealed with clay and placed in sterilized containers for transport to the lab. Blood was transported to the laboratory usually within 60 min. of collection for the best results during immune assays [14]. The microbicidal assay produces an index of the capacity of the blood, measuring how well blood can kill microbes after incubation/number inoculated (10 µL of working culture per 100 µL of media) and then immediately plated. Negative controls in which no microbes were added were also prepared. Microbicidal activity of the blood was defined as the percent of the inoculum killed which equal 1 (visible microbes after incubation/number inoculated) [14].

Discussion

These data show evidence of life-history trade-offs. At ND-6, nestlings possessed little to no immunity (Fig. 1) while presumably exceedingly considerable amounts of en- ergy were allocated to growth [5]. At ND-12, Tree Swallow nestlings showed increased immu- nity over that of ND-6 nestlings (Fig.1) and weighed as much or more than adults and then dropped in weight as they began to al- locate their energy into feather growth [16, 15]. As they transitioned to ND-18, there was another significant increase in immu- nity, further suggesting differences in energy allocation to immunity appeared to differ between the three stages of development in nestlings as well as between ND-18 nestlings and adults. Though body weight remained nearly the same, percent lysis (Fig. 4) was sig- nificantly less in ND-18 than in adults. This suggested that a large amount of energy was still being used by ND-18s for wing growth, thus less was devoted to developing immu- nity, further suggesting differences in energy allocation with an immunological cost dur- ing growth and development, which is con- sistent with findings in previous studies [19, 5]. In addition to trade-offs between growth and immunity development, it has been shown that high body temperatures reduce both tissue mass and humoral immunocompetence [20] in both adult female Barn Swallows, Hirundo rusti- ca, and her nestlings, showing decreased im- munity (Fig. 2) and adult female body mass increased as the nestlings grew, per capita predation rate with limited resources [21]. This was supported with the findings of Nordling et al., [22] which showed that female Collared Flycatchers, Ficedula albicilla, rearing enlarged broods, had lower humoral immunocompetence and increased parasit- ism, and that parasitism was associated with a reduction in survival probability. Because there is strong selection pressure to develop and control infection by pathogens and para- sites, a reduction in immunocompetence might lead to an increased risk of infection, thus reduced fitness and chances of survival [7].

Finally, there are data suggesting that leave- ing the nest before the nestlings are able to be part of the normal behavioral repertoire of altricial nestlings [22] and is associated with adaptations in their ontogeny and metabolism as brood size increases. Meineck Pia- n, Aethusa pratensis [22], suggests patterns of growth in nestlings with directional selec- tion for high growth rates, where the mean growth rate of nestlings in entirely depre- cated broods was lower than the population mean while that for partially depredated broods was higher [5]. At ND-18, the least growing nestlings was reared by reducing the ratio of growth for rapid growth in altricial bird species, in- cluding Tree Swallows. In summary, I studied the development of the innate immune response in nestling Tree Swallows at different stages of development via blood samples using microbicidal assays. I found that there is strong selection pressure to de- velop a competent innate immune response, as expected, there was a steady increase in the ability of the innate immune system to kill many of the important components of the innate immune system, with the exception of those birds that grew extremely fast. The number of nestlings was reduced by either nestling competition for food or by predation, or by a combination of both. Magrath [26] showed that the post- fledging survival of slowly growing young is low, suggesting that mortality due to lack of food and by predation were synergistic, yet both of these accounted for ND-18s and adults. 

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immunity in a free-living, altricial bird, the Tree Swallow. Microbicidal assays [14] were used to evaluate the innate immune function of nestlings at different stages of development, and I predicted that immune function would increase over the course of the nestling period.

Methods

Tree Swallows are a common, aerially foraging, passerine species distributed through- out North America that readily accept nest boxes. I tested nest boxes in a forest preserve in the campus of Grand Valley State University (GVUS) [42*57 N, 89*55 W], Ottawa County, Michigan in June and early July, 2009. Nestles were monitored closely to determine clutch completion date, exact hatch date, and, therefore, exact nestling age.

I collected blood samples from 36 nest- ling pairs by venipuncture occurring at the brachial vein in the wing using very small blood volumes (< 100 µL) that increased reproductive effort reduces energy allocation to immunity, as clearly seen with the ND-6 nestlings. Juveniles appeared to allocate more energy towards growth than adults and then dropped in weight as they began to allocate their energy into feather growth [16, 15]. 136 137GVSU

Min. of handling because the stress hormone nearest 1 mm using a ruler with a stop fixed ND-18 nestlings, I measured nestling mass into feather growth [16, 15]. In addition, on or more than adults and then dropped in weight as they began to allocate their energy into feather growth [16, 15]. 136 137GVSU

Discussion

These data show evidence of life-history trade-offs. At ND-6, nestlings possessed little to no immunity (Fig. 1) while presumably expending considerable amounts of energy towards growth (Fig. 2). ND-12 Tree Swallow nestlings showed increased immunity over that of ND-6 nestlings (Fig. 1) and weighed as much or more than adults and then dropped in weight as they began to allocate their energy into feather growth [16, 15]. As they transitioned to ND-18, there was another significant increase in immunity as the white blood cell mass increased to during phagocytosis). It was expected, there was a steady increase in the growth rate of nestlings in entirely depredated birds. Finaly, there are data suggesting that leaves a trade-off may reflect the balance between the innate immune response in nestling Tree Swallows at different stages of development via blood samples using microbicidal assays. Lysis increased as hatch time increased, suggesting that a greater number of nestlings was being used by ND-18s for wing growth, thus less was devoted to developing immunity, further suggesting differences in energy allocation with an immunological cost during growth and development, which is consistent with findings in previous studies [19, 5]. In addition to trade-offs between growth and immunity development, it has been shown that there is much less variation in the humoral immuneocompetence [20] in both adult female Barn Swallows, Hirundo rustica, and her nestlings, showing decreased immune competence as nest size increased as 10 eggs per nest, per capita feeding rate with limited resources [21]. This was supported with the findings of Morley et al. [22, 18] that mean mass = 21.3 g, P < 0.001. Though body weight remained unchanged between ND-18 and ND-12 for adult mass = 20.8 g, n = 79), wing length was significantly shorter (second female = 110.3 mm ± 2.6 mm, z = 12.2 mm, n = 77), second year male = 112.6 mm ± 4.9 mm, z = 21.6; second year female = 116.7 mm ± 4.2 mm, z = 5.1, n = 51; after incubation samples were removed from the incubator, vortexed, and duplicated, 50 µL aliquots were pipetted onto agar plates, spread, inverted, and incubated at 37º C for 24 hours. E. coli colonies were counted after 24 hours. The number of microbes in the initial inoculums were determined by diluting microbes in media alone (10 µL of working culture per 100 µL of media) and then immediately plated. Negative controls in which no microbes were added were also prepared. Microbicidal activity of the blood was defined as the percent of the inoculum killed which equal 1 (visible microbes after incubation/normol inoculated) [14].

Results

Total numbers of agar plated E. coli bacte-ria were measured in nestlings at ND-6 (n = 10), ND-12 (n = 16), and ND-18 (n = 18) for rapid growth in altricial bird species, in-cluding the Tree Swallow.

In summary, I studied the development of the innate immune response in nestling Tree Swallows at different stages of development via blood samples using microbicidal assays. Lysis increased as hatch time increased, suggesting that a greater number of nestlings was being used by ND-18s for wing growth, thus less was devoted to developing immunity, further suggesting differences in energy allocation with an immunological cost during growth and development, which is consistent with findings in previous studies [19, 5]. In addition to trade-offs between growth and immunity development, it has been shown that there is much less variation in the humoral immuneocompetence [20] in both adult female Barn Swallows, Hirundo rustica, and her nestlings, showing decreased immune competence as nest size increased as 10 eggs per nest, per capita feeding rate with limited resources [21]. This was supported with the findings of Morley et al. [22, 18] that mean mass = 21.3 g, P < 0.001. Though body weight remained unchanged between ND-18 and ND-12 for adult mass = 20.8 g, n = 79), wing length was significantly shorter (second female = 110.3 mm ± 2.6 mm, z = 12.2 mm, n = 77), second year male = 112.6 mm ± 4.9 mm, z = 21.6; second year female = 116.7 mm ± 4.2 mm, z = 5.1, n = 51; after incubation samples were removed from the incubator, vortexed, and duplicated, 50 µL aliquots were pipetted onto agar plates, spread, inverted, and incubated at 37º C for 24 hours. E. coli colonies were counted after 24 hours. The number of microbes in the initial inoculums were determined by diluting microbes in media alone (10 µL of working culture per 100 µL of media) and then immediately plated. Negative controls in which no microbes were added were also prepared. Microbicidal activity of the blood was defined as the percent of the inoculum killed which equal 1 (visible microbes after incubation/normol inoculated) [14].

Responsible Conduct of Research—The methods used in this study were in ac-cordance with suggestions for the ethical handling of wild birds [18]. We obtained permission to conduct this study from the GVUS Inves-tigational Animal Care and Use Committee (IACUC).

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Figure 1. Mean percent lysis (± SE) measuring the ability of the blood to kill bacteria *E. coli* at different stages of development in nestling Tree Swallows (ANOVA, $F = 12.25$, df = 2,50, $P < 0.001$).

**E. coli Lysis**

![Graph showing E. coli lysis over nestling days ND6, ND12, and ND18.](image)

Figure 2. Measurements of wing chord and mass showing percent lysis assessing the ability of the blood to kill bacteria *E. coli* for each nestling day 12 Tree Swallow sampled.

**ND 12**

![Graphs showing wing chord and mass over percent lysis for ND12.](image)

Figure 3. Measurements of wing chord and mass showing percent lysis assessing the ability of the blood to kill bacteria *E. coli* for each nestling day 18 Tree Swallow sampled.

**ND 18**

![Graphs showing wing chord and mass over percent lysis for ND18.](image)

Figure 4. Mean percent lysis (± SE) measuring the ability of the blood to kill *E. coli* bacteria at different stages of development, including adults, in Tree Swallows (ANOVA, $F = 62.12$, df = 3,123, $P < 0.001$).
Figure 1. Mean percent lysis (± SE) measuring the ability of the blood to kill bacteria E. coli at different stages of development in nestling Tree Swallows (ANOVA, $F = 12.25, df = 2,50, P < 0.001$).

**E. coli Lysis**

![Graph showing mean percent lysis for different nestling days.

Figure 2. Measurements of wing chord and mass showing percent lysis assessing the ability of the blood to kill bacteria E. coli for each nestling day 12 Tree Swallow sampled.

**ND 12**

![Graphs showing wing chord and mass measurements for different percent lysis.

Figure 3. Measurements of wing chord and mass showing percent lysis assessing the ability of the blood to kill bacteria E. coli for each nestling day 18 Tree Swallow sampled.

**ND 18**

![Graphs showing wing chord and mass measurements for different percent lysis.

Figure 4. Mean percent lysis (± SE) measuring the ability of the blood to kill E. coli bacteria at different stages of development, including adults, in Tree Swallows (ANOVA, $F = 62.12, df = 3,123, P < 0.001$).
References


References


