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Peripheral Blood Leukocyte Counts in Welcome Swallow Nestlings

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ABSTRACT: It is unclear whether developmental trends in total leukocyte (WBC) and differential lymphocyte (PropL) counts in peripheral blood of altricial birds typically mirror the known ontogenetic increase in immunocompetence. We documented the development of leukocyte and lymphocyte numbers in peripheral blood of wild, altricial Welcome Swallow (*Hirundo neoxena*) nestlings. Nestlings had a mass-overshoot–recession growth profile. Hatchlings' mean WBC (7.94×10^9 cells/l) and PropL (0.65) were respectively 4× and ~1.7× the mean adult value. Both variables declined at a steady rate throughout nestling development and were 1.3× the mean adult value at fledging. Hatching WBC values that substantially exceeded those of adults could have reflected the parasite- and pathogen-rich nest environment of this species. The developmental declines in peripheral blood WBC and PropL were not inconsistent with an ontogenetic increase in specific immunocompetence; they are likely to have resulted mainly from an increase in the rate of leukocyte trafficking to vulnerable tissues and organs.

Key words: Altricial development, *Hirundo neoxena*, immunocompetence, leukocyte count, lymphocyte, peripheral blood, Welcome Swallow.

Avian hatchlings are exposed to many parasites and pathogens. They depend for protection on innate immunity and maternal antibodies obtained via the egg yolk, which have a relatively narrow, antigen-specific repertoire (Deeming, 2002; Pihlaja et al., 2006). Endogenous antibodies gradually replace maternal immunoglobulins, but acquired immunity takes several weeks or months to reach adult levels in galliform birds (Klasing and Leschinsky, 1998). However, our knowledge of the ontogeny of innate and acquired immunity in altricial birds contains surprising gaps (Apanius, 1998), with a particular deficiency concerning developmental trends in numbers and types of leukocytes in peripheral blood. Leukocytes are central in effecting innate and acquired immunocompetence in birds (Klasing and Leschinsky, 1998). Heterophils and lymphocytes are the most numerous and functionally significant leukocytes in adults, the latter being crucial in effecting antibody- and cell-mediated specific immunocompetence.

Leukocyte numbers and activity are not necessarily equivalent in birds (Salvante, 2006) and lymphocytes spend only a limited time in peripheral blood, so it is pertinent to examine whether developmental trends in the total leukocyte count (WBC) and proportional lymphocyte count (PropL) in peripheral blood mirror the age-related increase in immune function documented for growing nestlings and chicks of several species (Apanius, 1998; Pihlaja et al., 2006). We determined whether the developmental trend in WBC and PropL in peripheral blood of wild Welcome Swallow (*Hirundo neoxena*) nestlings in southeastern Australia mirrored this widely demonstrated ontogenetic increase in immune function. The WBC and PropL at hatching, asymptotic mass attainment, and fledging were examined to see how closely they approximated to adult values.

The study was conducted from September to December, 2006, at five sites in the Yarra Valley (37°40′S, 145°07′E) and one at Clayton (37°46′S, 145°07′E), Victoria, Australia. Welcome Swallows nested colonially at three sites and in small groups or as solitary pairs at the others. Their cup-shaped mud nests were attached to vertical concrete surfaces under bridges and on buildings. These swallows often produce two broods of three to five nestlings annually and their mean incubation (16 days) and nestling (23 days) periods are comparatively protracted (Simmons and Lill, 2006). Fledglings are comparatively mature and fly fairly proficiently.

Nestlings representing all stages of nestling development ($n=105$) and 30 breeding adults were each sampled once. Adults were mist-netted and uniquely banded before release; nestlings were temporarily marked by clipping one rectrix. Birds were weighed (±0.5 g) and a small blood sample was taken from the leg or wing by capillarity after venipuncture with a 27-gauge syringe needle. Blood samples for WBC measurement were transported on ice and analysed within 8 hr. The WBC (cells $\times$ 10^9/l blood) was determined in an Improved Neubauer hemocytometer, using 10 µl of blood diluted with Natt and Herricks solution (Campbell, 1995). The PropL was usually determined from three blood smears per bird made by the push-wedge method (Turgeon, 2005) immediately after collection, but a few birds yielded only enough blood for two smears. Smears were air-dried but not fixed, stained with Aviacolor® (Cytocolor Inc., Hinckley, Ohio) within 2 days of collection and examined under 100× magnification with a binocular microscope. Lymphocytes and other granulocytes were tallied until 50 cells had been counted. The error rate involved in making lymphocyte counts (i.e., the mean variance in values obtained from counts of three slides for each of six birds) was 7.55±1.25. Least squares regression analysis of developmental trends in WBC (log transformed), PropL, and body mass was conducted with R 2.4.1 (Gentleman and Ihaka, 2006). The regression equations are given in the legends to Figures 1, 2, and 3. The best fit to the mass-age curve was provided by a quadratic regression term, but we also fitted a piecewise linear regression composed of two intersecting straight lines using Oddjob version 5.1 (Dallal, 1989). Nestling age was either determined from hatching dates or estimated from body mass using our data for known-age nestlings and those of Simmons and Lill (2006). Data are expressed as means±standard error.

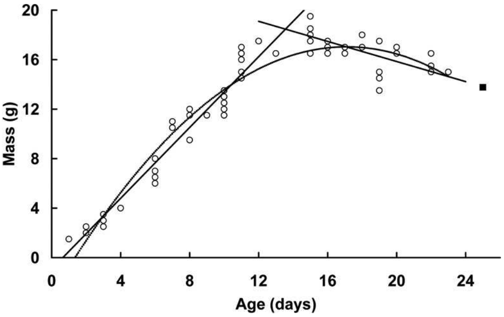

FIGURE 1. Body mass as a function of age in nestling Welcome Swallows. Dashed line is the quadratic regression and solid lines are the two linear regressions with the least difference in slope that fit the mass-age relationship. Quadratic regression equation: $\text{Mass}=-0.069(\text{age})^2+2.349(\text{age})-3.054$ ($n=74$, $r^2=0.944$, $F=602.7$, $P<0.001$). Linear regression equation for first phase: $\text{Mass}=1.43(\text{age})-0.917$ ($n=45$, $r^2=0.911$, $F=984.391$, $P<0.001$). Linear regression equation for second phase: $\text{Mass}=0.406(\text{age})+23.97$ ($n=29$, $r^2=0.024$, $F=25.745$, $P<0.001$). Mean adult mass shown by a black square (SE=0.159 g).

Nestlings exhibited a mass-overshoot–recession growth profile (Ricklefs et al., 1998), which is particularly apparent from the piecewise linear regression. Nestling mass increased 14.6-fold from hatching (estimated mean 1 g) to fledging (Fig. 1). It increased rapidly to a maximum value

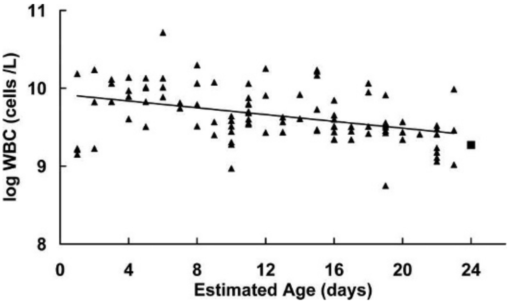

FIGURE 2. Linear regression of leukocyte count in peripheral blood on age of nestling Welcome Swallows. Regression equation: $\log \text{WBC}=-0.022(\text{age})+9.924$ ($n=101$, $r^2=0.161$, $F=18.98$, $P<0.001$). Adult mean value shown as a black square (SE=±0.03).

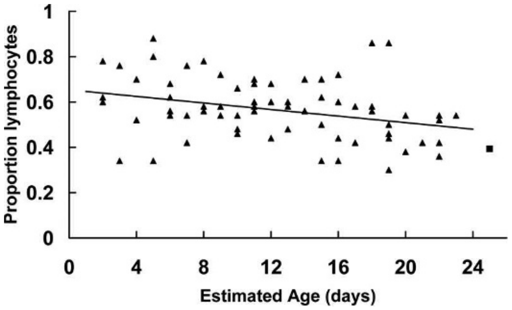

FIGURE 3. Linear regression of proportion of lymphocytes in peripheral blood on age of nestling Welcome Swallows. Regression equation: Proportion of lymphocytes $=-0.723$(age)$+65.342$ ($n=76$, $r^2=0.102$, $F=8.402$, $P<0.01$). Adult mean value shown as a black square (SE $=\pm0.02$).

~19% greater than mean adult mass (13.8±0.2 g) on day 14 (i.e., after ~61% of the nestling period had elapsed). It then slowly decreased, although estimated mean fledging mass was still ~6% greater than adult mass. Nestling age explained 94% of developmental variation in body mass, but only 2% of the limited variation during the mass recession phase.

Mean hatching WBC (7.94×10^9 cells/l) was nearly 4× greater than the mean adult value of 2.02×10^9 cells/l. It decreased significantly and steadily during nestling development, but age explained only 16% of the variation (Fig. 2). At fledging it was only 2.63×10^9 cells/l or just 1.3× the mean adult value. Mean hatching PropL (0.65) was ~1.7× the mean adult value (0.39±0.02). It decreased significantly and constantly during development (Fig. 3), although nestling age accounted for only 10% of the variation. At asymptotic mass attainment (~day 14), it was still ~1.4× the mean adult level and at fledging ~1.3× that level.

Avian endogenous immune function is relatively limited at hatching, but increases during development (Apanius, 1998; Klasing and Leschinsky, 1998). This raises the issue of why the Welcome Swallow's peripheral blood WBC and PropL were substantially greater than adult levels at hatching and declined during nestling development.

Swallows' nests often have particularly high parasite loads (Møller, 1990; Rogers et al., 1991; Christe et al., 2001). Breeding success is reduced more by parasites in the colonially breeding than in the solitarily species and nestlings' cell-mediated immune responsiveness is also greater in the colonial species (Møller et al., 2001). Therefore, because most swallows in this study were colonial breeders, they could potentially have been subject to strong natural selection for early expression of innate immune competence (Apanius, 1998). This hypothesis might explain the relatively high peripheral blood WBC in hatchlings and could be explored by comparing innate immunocompetence levels and peripheral blood differential heterophil counts of hatchling Welcome Swallows in 1) colonially and solitarily breeding populations and 2) with other avian species that are less exposed to nest parasites and pathogens. However, the hypothesis fails to adequately account for the higher peripheral blood PropL in hatchlings than in adults, because lymphocytes are primarily involved in effecting acquired immunocompetence.

The few nestling-adult comparisons of WBC and PropL published for other birds (Fairbrother and O'Loughlin, 1990; Work, 1996; Pap and Márkus, 2003) mirror the disparity between nestlings and adults recorded in Welcome Swallows. Moreover, similar ontogenetic declines in these variables also occur in humans (Lisse et al., 1997) and several other mammals, a generality that gives us confidence that the decrease observed in the swallows was not simply a product of misidentification of early-stage lymphocytes.

Whether or not a developmental decrease in peripheral blood leukocyte counts proves to be widespread among altricial birds, it is not inconsistent with a developmental increase in acquired immunocompetence. Only a small proportion of an altricial bird's leukocyte population is present in the peripheral bloodstream at any given time (Apanius, 1998), with a much greater proportion occurring in various organs, including the lymphoid organs. In domestic pigeon (*Columba livia*) hatchlings, WBC and PropL in the lymphoid organs are well below adult levels and increase in parallel with improving specific immunocompetence during early development (Selvaraj and Pitchappan, 1988). Despite the comparatively low PropL in the pigeon hatchling's lymphoid organs (e.g., 65% of splenocytes), lymphopoiesis during development is clearly sufficient to produce the vast number of lineages and clones required for effective humoral immune function by 3–4 mo of age in this species (Selvaraj and Pitchappan, 1988). Documenting WBC and PropL in the lymphoid and other organs during Welcome Swallow nestlings' development could establish whether an analogous scenario occurs in this species.

The increase in WBC and PropL in the lymphoid organs during early development in pigeons is presumably facilitated mainly by lymphopoeisis continuing well beyond hatching, as in precocial chickens (Klasing and Leschinsky, 1998). The most likely explanation for the decrease in peripheral blood leukocyte counts in Welcome Swallow and other altricial nestlings early in development is an increase in the trafficking rate (Dhabhar et al., 1995) to vulnerable organs and tissues. Later in nestling development, as mature acquired immune function is approached, the rate of lymphopoiesis should decrease, which may augment the decline in peripheral blood leukocyte counts.

Ricklefs et al. (1998) argued that once body mass overshoots adult mass relatively early in development in birds exhibiting a mass-overshoot–recession nestling growth profile such as that of the Welcome Swallow, functional maturation dominates over growth because simultaneous growth and physiologic maturation of tissues are incompatible. We did not determine when fully mature acquired immunocompetence was attained in Welcome Swallows. Nonetheless, the fact that peripheral blood WBC and PropL declined toward the typical adult level throughout nestling development might indicate that maturation of immunocompetence was not strongly influenced by the attainment of mature body size when only ~60% of the nestling period had elapsed. This could be tested by applying appropriate challenge techniques (Norris and Evans, 2000) to swallow nestlings before and after the attainment of asymptotic mass.

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