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The costs of immunity

Kirk C. KLASING*
Department of Animal Science，University of California，Davis，California 95616，USA

Abstract The costs of development of the immune system come primarily from the expenditure of energy to fuel the inefficient process of developing antigen recognition diversity in B and T lymphocytes and to provide substrates (e.g.，amino acids and lipids) for the initial burst of leukopoiesis needed to supply the late embryo and hatchling with leukocytes. The costs of maintaining the immune system are related to allocating nutrients for the continued production of leukocytes，immunoglobulin，and other plasma proteins to replace those lost during normal turnover of cells and extra-cellular proteins. The costs of using the immune system to thwart the invasion of potential pathogens come in two primary forms. First，there are losses in tissue function that result from damage incurred when leukocytes engage their effector mechanisms and damage tissue integrity and host cell viability (collateral damage). Secondly，there are nutritional costs in mobilizing the responding cell types and fueling their effector functions. The primary cost of an authentic pathogen challenge is in the systemic acute phase response，especially recruitment of the liver，to assist the immune system by producing protective proteins [*Acta Zoologica Sinica* 50 (6): 961-969，2004].
Key words Immunity，Innate，Adaptive，Costs，Maintenance，Developmental

免疫的代价

Kirk C. KLASING*
Department of Animal Science，University of California，Davis，California 95616，USA

摘 要 免疫系统发育的代价主要是为B淋巴细胞和T淋巴细胞发育抗原识别多样性时的低效率过程提供能量，为开始形成白血球提供基质（如氨基酸和脂肪），并为胚胎和雏鸟提供白细胞。维持免疫系统的代价与持续产生白细胞、免疫球蛋白以及其它血浆蛋白时的营养分配有关，这些血浆蛋白用于取代在正常的细胞代谢中丢失的蛋白质以及细胞间的蛋白质。利用免疫系统阻碍可能的病源体入侵的代价有两种主要形式。第一，由于白细胞参与效应子机制时发生的损害、组织整合性以及寄主细胞的存活力的损伤，组织功能有一些损失。第二，在活化相应类型的细胞并产生其效应子作用时存在着营养耗费。病源体挑战所带来的主要代价是系统的急性期反应（特别是肝脏的复原）通过产生保护性的蛋白质来帮助免疫系统 [动物学报 50（6）：961-969，2004]。
关键词 免疫 先天 适应性 代价 维持 发育

1 Introduction

Vertebrates shield themselves against virus，bacteria，and eukaryotic parasites by investing in a multilayered and complex system of protection: the immune system. Among birds，there is marked interspecies and intraspecies variation in susceptibility to challenges by pathogens. Given that there are costs attributable to the processes providing protection，it can be expected that much of the variability in pathogen susceptibility is due to differing investments in the size and quality of the immune system. Evidence from genetic selection studies (Siegel et al.，1982b; Parmentier et al.，1995; Parmentier et al.，1996; Qureshi and Havenstein，1994; Martin et al.，1990) and from interspecies and intraspecies comparisons (Moller et al.，2003; Martin et al.，2003; Tella et al.，2002) support this contention. Studies in physiological ecology and theoretical immunology often refer to the "costs" in terms of resources，such as the energy needed to mount an immune response，or the trade-offs between immunocompetence and other nutrient-requiring functions (Read and Allen，2000). An accounting of these costs should be precise for birds，for these theories and arguments to have a sound quantitative basis. However，the costs of immunity have rarely been described with precision，so the importance of trade-offs between immunity and

* Corresponding author. E-mail: kcklasing@ucdavis.edu

other physiological processes remains obscure. Given that investment in self-maintenance, especially immunity, is presumed to be an important constraint in the diversification of life-histories (Ricklefs and Wikelski, 2003; Klasing and Leshchinsky, 1999), a greater appreciation of the costs of immunity is needed.

The immune system has many layers and is pervasive within most physiological processes of the body. All cells have mechanisms that provide self-maintenance and thwart infectious challenges. For example, foreign RNA is silenced by an intricate molecular mechanism that minimizes the ability of viruses to commandeer the translational machinery of a cell (Plasterk, 2002). Though all body cells have defensive pathways, leukocytes are the professional cells of the immune system and are responsible for detection of pathogens, production of molecules to eliminate the pathogen, and alerting nervous, endocrine and other regulatory systems of infectious challenges. Leukocyte functions are typically divided into innate and adaptive components. Innate immunity is mediated predominantly by phagocytes and is crucial for defense against novel pathogens. Adaptive immunity is mediated predominantly by lymphocytes, and requires four or more days of activation before contributing significantly to protection against novel pathogens. Notably, subsequent challenges by a pathogen are quickly and effectively thwarted by adaptive immunity because of its memory capabilities, whereas innate immunity is no more effective against a second challenge than it was against the first.

2 Estimating the cost of immunity

As in all physiological processes, the nutritional costs of immunity are best subdivided into three components: those of developing the immune system, those of maintaining that system in working order, and those involved in use of the system to thwart a parasitic invasion. Development of the immune system is defined as the initial differentiation and selection events in the thymus and bursa that generate a diverse pool of lymphocytes expressing thousands of unique pathogen receptors needed to identify potential pathogens. Development also includes the clonal proliferation of all leukocyte populations and their occupation of epithelia and secondary lymphoid organs. The maintenance state is defined as a quiescent immune system engaged in routine functions such as clearing apoptotic cells that result from normal cell turnover. The use of the immune system is defined as a response to a foreign organism or molecule designed to contain or deactivate it.

Use of the immune system includes phagocytosis, the acute phase response, proliferation of antigen-specific lymphocytes, antibody production, and cell-mediated cytotoxicity. Current theory predicts that there is a tradeoff between inductive versus constitutive types of protection (Shudo and Iwasa, 2002; Schmid-Hempel and Ebert, 2003). Maintaining constitutive levels of protective cells and proteins at protective levels should provide the most rapid and effective defense, but it has high nutritional costs. Alternately, a low level of constitutive production of protective factors, with rapid induction following a pathogen challenge, is predicted to have cheaper cost if lower protective value. From this viewpoint, constitutive production of leukocytes and protective proteins is a maintenance activity, while the increase due to a challenge is considered use of the system.

The cost of immunity can be estimated directly or indirectly. Direct estimates are derived from population dynamics, energy expenditure, and rates of material accretion by leukocytes and other cells that contribute to immunity. Indirect estimates can be obtained by examining the extent of trade-offs in competing physiological processes that occur due to immune processes. Ultimately, our confidence in the cost of immunity depends on the congruence of direct and indirect estimates.

Quantitative measurements of leukocytes and their products typically require monoclonal antibodies and other reagents that are species-specific and unavailable except for agriculturally important species. For this reason, direct estimates of the cost of immunity in the Aves are available only for chickens *Gallus gallus*. However, indirect estimates are not particularly reagent-dependent and can be obtained across avian orders; though to date, most of the work has also used the Galliformes, especially the chicken and Japanese quail *Coturnix coturnix japonica*.

2.1 Direct estimates

The nutritional costs of many physiological processes have been well defined. For example, the resting metabolic rate of many hundreds of avian species has been measured and often re-measured. The additional costs to support activity, thermoregulation, growth, molt, and egg production have likewise been detailed with precision. Data on the rate of tissue accretion, the nutrient composition of tissues, and the rates of macromolecule turnover have provided a firm scientific basis for estimating the nutritional demands of growth, molting, and egg production (Klasing, 1998b; National Research Council, 1994). Unfortunately, a similar quantitative foundation is mostly lacking for the estimation of the nutritional resources needed for immunity. This is probably due to the complexities and uncertainties surrounding the process. Immunity is mediated by at least ten lineages of leukocytes that are located diffusely throughout the body. Nobel prizes are awarded almost annually for

fundamental advancements in our understanding of the immune system and its regulation. Clearly, it is difficult to evaluate, quantitatively, physiological systems that are incompletely understood and hard to locate.

Furthermore, the diverse types of challenges (e.g., viral, bacterial), the variable intensities and duration of the challenge (acute vs. chronic), and the differences in immune responses triggered by pathogens (e.g., innate, cell-mediated, humoral) make generalizations difficult. Nevertheless, several attempts have been made to determine the cost of immunity, including attempts to sum factorially the costs of the various processes of immunity (Lochmiller and Deerenberg, 2000; Klasing and Calvert, 1999; Cohn and Langman, 1990; Langman and Cohn, 1993). In our attempt (Klasing and Calvert, 1999), amino acids were used as the currency for estimating costs because the immunology literature is relatively replete with estimates of rates of protein accretion (e.g., antibody or acute phase protein synthesis) and cell proliferation; but there is a dearth of information on the energetics of these processes. Lysine, in particular, is used as the nutrient of choice for factorial summation of the costs of immunity because this amino acid has few uses other than as a substrate for protein synthesis.

Because we do not yet know all of the processes responsible for immunity, factorial summations necessarily underestimate the total costs. While incomplete and presumably providing an underestimate of the total cost of immunity, direct estimates provide important perspectives on the relative costs of different components of the system. As described below, the systemic acute phase response that accompanies an innate immune response appears to be the most expensive component of immunity in young chickens (Table 1).

Table 1 Relationship between types and costs of immune responses

Parameter	Developmental time	Developmental costs	Maintenance costs	Nutritional costs of use	Effectiveness		Pathological costs of use
					Novel challenge	Repeated challenge	
Non-specific immunity, including acute phase response	short	low	medium[1]	very high[1]	good	good	very high
Specific, lymphocyte-mediated immunity	long	very high	low	low	poor	excellent	variable[2]

1. Constitutive production of protective levels of complement, natural antibodies, and other broad-spectrum defensive proteins is considered as a maintenance cost. During an infectious challenge, rapid secretion of these proteins plus acute phase proteins has a high cost.
2. Many of the pathological actions of specific immunity occur when immunoglobulins or T-lymphocytes activate macrophages and other cells of the non-specific system.

2.2 Indirect estimates

Because of the myriad of cell types, effector mechanisms and locations of the immune system, indirect estimates of the resources needed for protection have utility. Implicit in this approach is that the costs needed for immunity impinge on the nutrients available for other physiological processes. For example, the energy used by leukocytes during a response to a pathogen presumably siphons away energy for growth, reproduction etc. The decrease in energy deposited in growing or storage tissues that occurs during such a protective immune response should be proportional to the amount of energy utilized by that immune response. While indirect estimates of the costs of immunity are not particularly useful for identifying the relative contribution of various immune processes to the total cost, they circumvent many of the problems associated with our incomplete understanding of the process of immunity and availability of reagents to quantify the processes.

Though energy expenditure is the preferred currency for the measurement of many life history characteristics, it has not always provided reliable results for studies on the cost of immunity. This is because there are many behavioral changes that accompany the immune response to pathogens which confound interpretation of changes in energy expenditure. For example, the acute phase response that accompanies immune responses to pathogens markedly decreases activity levels compensating for increases in energy expenditure attributable to immunity; the net result is little change in energy expenditure (Parmentier et al., 2002; Johnson et al., 1993). Indirect estimates of the developmental costs of immunity are not currently available, but indirect estimates for maintenance and use of the immune system have been made.

3 Costs of immunity development

The innate and adaptive arms of the immune system follow very different developmental schemes and, consequently, have very different developmental costs. In general, development of the innate immune system is gradual and efficient and has no obvious costs that distinguish it as being uniquely expensive.

Development of the adaptive immune system occurs through a notably inefficient process during a limited window of time, imposing a uniquely high cost.

3.1 Adaptive immunity

The receptors that lymphocytes utilize to recognize pathogens are generated via a series of semi-random recombination and point mutations in their coding genes within the thymus and bursa (Reynaud and Weill, 1996). This semi-random diversification is necessary to permit the immune system to recognize diverse pathogens and diminish evolution of their resistance. Diversification of antigen receptor genes typically results in ineffective receptors, which signal the deletion of the lymphocyte involved. Only rarely does a recombination event result in a functional antigen recognizing capability that does not recognize self-antigens too strongly. In these instances, that lymphocyte divides and migrates into the periphery where it populates lymphoid tissues and epithelia. In chickens, approximately 90% of developing B-lymphocytes and 95% of developing T-lymphocytes are non-functional and are deleted in the bursa and thymus, respectively (Reynaud and Weill, 1996; Lassila, 1989). This is the most inefficient developmental process occurring in the embryo and hatchling, and presumably it is energetically very costly.

Rates of protein synthesis and accretion within chicken bursa and thymus, rates of growth of the thymus and bursa, and the rate of export of lymphocytes to the periphery permit estimates of the developmental costs of adaptive immunity. Rapid B-cell development starts at about E-14 and continues to about 4 weeks after hatching. The bursa expands from about 0.05% of body weight at E-14 to 0.45% of body weight at 3－4 weeks after hatching, when it reaches its maximum size (Betti and Sesso, 1989; Mercer-Oltjen and Woodard, 1987). At 4 weeks post-hatching, the bursa exports 5×10^8 cells per day (Paramithiotis and Ratcliffe, 1994), representing 0.04% of body weight. The total lysine needed for expansion of bursa mass and cell export is 837 μmol. During this same period, 75 950 μmol of lysine are accreted in the whole body. Consequently, B-cell development accounts for about 1.10% of the lysine accreted in a growing chick (Table 2). The overall kinetics of thymus growth and T-lymphocyte migration to the periphery approximates that of B-lymphocytes; and the proportion of lysine accreted for development of the adaptive immune system is estimated to be about 2.2% (Klasing and Calvert, 1999). The rate of protein synthesis in the thymus and bursa are 2.4 and 3.6 times the whole body rate (Klasing and Austic, 1984) and, correcting for relative organ size, development of lymphocytes accounts for about 3% of the total daily energy expenditure used for protein accretion in a 4-week-old chick.

Table 2 Daily rate of protective processes and growth in young chicks[1]

Process	Normal		LPS challenged	
	Production (mg/kg/d)	Cost (μmol lysine/kg/d)[2]	Production (mg/kg/d)	Cost (μmol lysine/kg/d)[2]
Leukopoiesis in all tissues	650	45.5	1 300	90.9
Immunoglobulin synthesis[3]	114	65.6	121	69.6
Acute-phase protein synthesis	~0[2]	~0	710	386
Total for immunocompetence	764	111.1	2 131	546.5
Body weight gain[3]	85 000	5 950	72 446	5 212
Lysine intake	–	9 520	–	8 311
% of intake used for immune processes		1.17		6.71
% of intake used for growth		62.50		62.70

1. From Klasing and Calvert (2000). LPS: Lipopolysaccharide.
2. Constitutive production of complement and other defensive proteins is not included in this analysis because their synthetic rates are not yet available.
3. For 2-week-old chickens.

Current theory on the diversification of the B and T cell receptor repertoire predicts that the number of lineages of functional lymphocytes that must be generated is independent of body weight (Cohn and Langman, 1996; Langman and Cohn, 1993; Cohn, 2000). This is because small birds are exposed to the same milieu of viruses, bacteria, and other parasites as large birds and must be able to recognize the same number of foreign antigens. Thus all birds, regardless of size, are predicted to have similar absolute costs for diversifying their lymphocyte repertoires. Clearly, this cost represents a much larger investment for small birds than large. Presumably the only way that small birds can cope with these developmental costs is by either spreading them out over a long period of time or by accepting a smaller repertoire and poorer

capacity to recognize pathogens. Theoretically, birds smaller than 4 g cannot have a complete repertoire of lymphocytes because they lack sufficient space in their bodies for the number of cells required in each lineage to establish effectively protective levels of immunity.

3.2 Innate immunity

Cells of the innate immune system (e.g., macrophages and heterophils) utilize pattern-recognition receptors that are coded in the genome and do not require diversification or selection. As progenitor cells proliferate, all daughter cells are functional, express a full repertoire of pathogen-recognition receptors, and have equal utility in combating parasites.

The stem cells that give rise to cells of the macrophage-phagocyte system are present early in development and proliferate slowly, populating lymphoid organs as they are formed (Kent, 1961). Because of such straight-forward development, nutrients are needed uniformly during embryogenesis and are proportional to the number of innate immune cells in the embryo and hatchling. The number of granulocytes, monocytes and macrophages reach adult densities at about 1 week of age in chickens (Mast and Goddeeris, 1998; Jeurissen et al., 1992; Jeurissen et al., 1994), and account for 0.18% (14.4 μmol) of the total of the 8.05 mmol of lysine accreted from E6 to 7 days of age (Table 2). Thus amino acid costs for development of the innate immune system appear to be relatively low. This simple and efficient development scheme, with all daughter cells competent, suggests that the energy requirements for this process would be low.

A hen makes a large investment in the innate immunity of her eggs and hatchlings. Chickens incorporate about 200 mg of immunoglobulin (IgY) into each egg yolk (Kowalczyk et al., 1985), which is about 3.8% of the egg's lysine. Furthermore, the antimicrobial proteins lysozyme, ovotransferrin, ovomucin, and avidin comprise about 25% of the egg white and function to protect the developing embryo (Romanoff and Romanoff, 1949).

4 Costs of immunity maintenance

The vast majority of lymphocytes in a healthy bird are "at rest" and are among the least metabolically active cells in the body. Their content of cytoplasm and RNA is exceptionally small, and rates of antibody secretion are negligible. Similarly, monocytes are not especially metabolically active unless they encounter a pathogen and are activated to differentiate into macrophages. A small fraction of B-lymphocytes differentiates into plasma cells which constitutively secrete immunoglobulin. The pool of constitutively secreting plasma cells includes lineages that have previously responded to a pathogen and lineages of B-1 cells that produce "natural antibody" that is especially important in constraining commensal microflora and clearing endogenous antigens. This constitutive secretion of immunoglobulin should be counted as a maintenance cost.

In the healthy adult, the immune system consists of a set of re-newing cell populations which continuously produce and lose cells in dynamic equilibrium. At maintenance, many of the cells of the immune system are relatively long-lived, with the exception of neutrophils. Replacement lymphocytes in immunologically mature animals result from the replication of lymphocytes in the existing pool. Consequently, each daughter cell is competent and the process of maintaining lymphocyte numbers is efficient. The liver is the primary accessory organ of the immune system and constitutively produces complement and other accessory proteins.

4.1 Direct estimates

The costs for maintaining the cells of the immune system can be put into perspective by examining the contribution of leukocytes and their effector molecules to body mass or body lysine (Table 2). The cellular elements of the immune system of an adult chicken contribute slightly less than 1% of the body weight. The primary effector protein in the body is Ig. In the chicken, total IgY, IgM and IgA (Leslie and Clem, 1970) comprise 0.13% of body weight and about 1.05% of total lysine content.

Though most leukocytes are very long lived, heterophils migrate into the intestines and IgA is secreted into the epithelia. At present, the cost of these losses is not known. Therefore, it is instructive to estimate the maintenance needs for the immune system from the rate of cell and Ig synthesis, assuming zero re-utilization of nutrients during replacement. The normal rate of immunoglobulin synthesis in chickens is about 0.025% of body weight per day (Leslie and Clem, 1970), which is 1.9% of the total lysine used by a 2-week-old chick. Leukopoiesis accounts for 0.76% of lysine use in a young chick. Based on serum concentrations and half-life estimates, it is likely that the amino acid demands for the synthesis of complement and other accessory proteins are an order of magnitude lower than that of Ig. Thus, the use of lysine for maintaining the immune system of a young chicken is likely to be less than 3% of the total.

4.2 Indirect estimates

Indirect estimates of the maintenance cost of immunity can be drawn from experiments examining chicks grown in a sterile environment relative to chicks grown in a conventional, but pathogen-free environment. Young chickens that have not been exposed to bacteria and viruses have far fewer leuko-

cytes, lower rates of leucopoiesis, and convert about 5% more of their dietary nutrients into body tissue than chickens in a conventional environment (Coates, 1973). Likewise, feeding high levels of antibiotics or antibacterials decreases the numbers of leukocytes, especially along the intestinal epithelium and spares about 5% of daily nutrient needs (Roura et al., 1992; Humphrey et al., 2002). These indirect estimates are somewhat higher than direct estimates (5% vs 3%) but confirm that the immune system has a measurable cost even when it is not being used to thwart pathogens.

5 Costs of immunity use

Any discussion of the costs of using an immune system requires a statement of the obvious: the pathology and necrosis induced by pathogens in immunodeficient hosts is usually fatal. On the other hand, a maximal response of the immune system may also be fatal (e.g., anaphylaxis or septic shock). Descriptions of the pathology induced by inadequate or excessive immune responses are detailed in every textbook on avian medicine, but estimates of the nutritional costs of a successful and appropriate immune response are poorly characterized.

The survival of an animal following infection by a pathogen is determined by the relative rate of pathogen proliferation versus the current level of protective immunity and capacity to recruit additional protection. Defense against novel pathogens is clearly more costly than re-engagement of previously encountered pathogens. The following analysis considers the costs of defense against a novel bacterial challenge. Most challenges begin with an influx of monocytes and heterophils from the blood to the site of infection and their secretion of effector molecules, such as reactive oxygen intermediates, lysozyme, complement and defensins. These phagocytes must be replaced by increased output from leukopoietic areas of bone marrow. Stimulated leukocytes release interleukin-1β (IL-1β), IL-6, tumor necrosis factor-α, and γ-interferon. These cytokines orchestrate a systemic acute phase response that includes fever, anorexia, and recruitment of the vast immune defense capabilities of the liver, especially the secretion of acute phase proteins (Parmentier et al., 1993; Klasing, 1998a; Adler et al., 1998; Johnson, 1997; Johnson et al., 1993). Over the next few days, B and T lymphocytes that recognize antigenic determinants on the pathogen begin to proliferate. The responding lymphocytes form germinal centers in nearby lymphoid tissues where affinity maturation of Ig occurs. After about a week, plasma cells begin to produce protective levels of Ig, and T-cytotoxic lymphocytes seek out infected host cells and kill them.

Most novel pathogens induce strong innate and adaptive immune responses, and costs of immunity must take both into account. Experimentally, many investigators use purified antigens like KLH, BSA or SRBC, which do not stimulate robust innate immune responses and are poor model systems for true infectious diseases. However, reagents that selectively stimulate either a B-lymphocyte response or a T-lymphocyte response are instructive in itemizing the specific costs of various arms of the immune system.

5.1 Direct estimates

The costs of using the immune system are equal to the increase in cell proliferation and secretion of effector molecules triggered by a pathogen (Table 2). Following a successful immune response, the number of leukocytes and levels of effector molecules return to normal and the nutrients left over are presumably redirected to other uses.

5.1.1 Innate responses The infiltration of leukocytes during the first day of a simulated peritoneal infection results in the accumulation of about 1.5×10^8 macrophages and heterophils per kg body weight (Golemboski et al., 1992; Klasing, 1998a; Sabet et al., 1977). This is equivalent to about 0.0025% of total body weight. In large mammals, fever has a considerable energetic cost, but in chickens and other birds, a net change in energy expenditure due to fever is not normally observed because of compensatory decreases in other expenditures such as activity, growth or reproduction. The accretion of acute phase proteins (Barnes et al., 2002; Adler et al., 1998) is the single most significant use of amino acids during an immune response, accounting for 386 μmoles of lysine/kg/d or 4.6% of the lysine intake in a 3-week-old chicken. Clearly, the recruitment of liver action away from its normal functions in secreting nutrients to support growth or reproduction to aid immune defenses is the single most expensive component of immunity to pathogens that induce an acute phase response.

5.1.2 Adaptive responses Acute systemic infections in mammals causes a 2-fold increase in the rate of leukopoiesis in bone marrow (Elgert, 1996) to 0.06% of body weight per day; but corresponding information for birds is not available. The rate of synthesis of Ig specific for epitopes on pathogens increases remarkably during an infectious challenge, from levels that are often undetectable to effective concentrations of between 10 and 100 ng of Ig per ml of extracellular fluid (Cohn and Langman, 1990) in less than a week. The total amount of antigen-specific-Ig produced following hyperimmunization of complex antigens augments plasma Ig levels by about 25% in chickens (Leslie and Clem, 1970). Assuming that a similar increase in Ig occurs in all extracellular fluids, this represents an increase of about 28 mg of Ig per

kg body weight per day. In chickens challenged with a highly antigenic protein, the influx of lymphocytes into the spleen and the formation of germinal centers accounts for only about 0.01% of body weight (Humphrey and Klasing, unpublished observations).

In most challenges by novel pathogens, the innate response precedes the peak adaptive response and the expenses of the two arms are spread sequentially. Only in the case of persistent pathogens can both innate and adaptive arms of the immune system be expected to respond simultaneously at high levels. The above accounting indicates that the amount of lysine needed to support such a simultaneous response is 550 μmol/kg/d. If this quantity were to be used for growth, it would support the accretion of 7.8 g of body mass/kg of body weight.

5.2 Indirect estimates

Indirect measurements of the cost of a vigorous immune response have been obtained by exposing growing chicks to bacterial lipopolysaccharide (LPS). LPS induces an intense innate and adaptive immune response simultaneously by stimulating macrophages, B-cells and T-cells through specific LPS receptors. Under this model system, growth rate slows from 85 g/kg/d to 72.4 g/kg/d in young chickens (Klasing et al., 1987; Benson et al., 1993). Interestingly, known process in immunity can account for about 60% this loss (Table 2); and most of the remainder is due to anorexia.

The high cost of an acute phase response probably explains why a variety of avian species respond to challenges from live bacterial and viral pathogens, and sometimes even relatively benign parasites, with changes in body condition and energy metabolism that are much greater than can be accounted for by summation of the substrates needed for adaptive immune responses (Sheldon and Verhulst, 1996; Martin et al., 2003; Korver et al., 1997). Even benign antigens like red blood cells in sheep stimulate a mild acute phase response that can be measured as increased heat production, protein turnover, fever, and slightly decreased rate of growth (Cook et al., 1993; Klasing and Austic, 1984; Klasing et al., 1987; Siegel et al., 1982a). The idea that an immune response diverts nutrients away from growth, reproduction and other productive purposes needs to be put into context. A quantitative analysis of the processes involved indicates that anorexia and changes in nutrient use by the major organs of a bird, especially the liver, are primarily responsible and that use of nutrients by leukocytes is only a minor contributor.

Although the above analysis has focused on model systems that invoke vigorous immune and acute phase responses, frequent challenges of low intensity can, over long periods of time, result in diminished productivity. For example, the frequency with which the immune system responds to challenges by opportunistic microorganisms is inversely proportional to the rate of growth and the efficiency of dietary energy use for tissue deposition. Hatchling chickens housed in environments where they have frequent but low level challenges have greater numbers of leukocytes present along their epithelia; presumably the cost of sustaining this higher level of surveillance amounts to about 5% of daily nutrient use (Roura et al., 1992; Humphrey et al., 2002).

6 Conclusions and predictions

All avian species require an immune system that is sufficiently competent to thwart the continuous challenges from commensal microflora and occasional challenges by true pathogens. However, immunity comes at a cost that must be paid at the expense of other processes, and it is unlikely that any species, or individual, possess the maximum immunity possible. Presumably there is a continuum of levels of investment for immunity at the species level, and that factors related to environment and life history drive this variability. Our current view of the avian immune system is colored heavily by what happens in the chicken. The chicken immune system is very different from that of mammals. In the few instances were other avian species have been examined, albeit superficially, surprising diversity has been found. For example, ducks produce a structurally and functionally different IgY to that of chickens, and the production of this unique effector molecule affects the cost of immunity markedly (Higgins, 1996; Humphrey et al., 2001). There is reason to expect that even greater diversity will be found as attention is focused on other orders that have greatly different body sizes, life-histories, and phylogenies.

Given that each component of the immune system has a unique cost and benefit, it is likely that there is a wide variety of strategies for immunity (Ricklefs and Wikelski, 2003; Klasing and Leshchinsky, 1999). Each strategy may accentuate specific components of immunity and de-emphasize others. Developmental costs are uniquely high for lymphocytes, and the rewards of owning a robust repertoire may not be sufficient in some species. Recombination, gene conversion, and mutation processes necessary for generating a diverse repertoire of lymphocytes expressing unique antigen receptors takes several months to develop and are a major cost to the chicken in terms of both time and energy. Presumably, some species forgo this expenditure and have a narrow and incomplete repertoire of lymphocytes that would result in lower probabilities of recognizing some pathogens as foreign. At the other end of the contin-

uum are species that invest resources to develop a robust repertoire of lymphocytes that can recognize nearly all potential pathogens and provide maximal protection.

Current knowledge predicts that species with long life spans will invest more time and nutrients into developing a robust repertoire of lymphocytes (Ricklefs and Wikelski, 2003; Klasing and Leshchinsky, 1999). Species with a long life span and slow reproductive turnover will be disproportionally affected by mortality from the rare pathogens that cannot be recognized by their innate immune system. Additionally, the longer an animal lives, the less likely that a pathogen challenge will be novel and the greater the benefit of memory for specific, lymphocyte-mediated immunity. Thus long-lived species receive more benefit from specific immunity and are more likely to invest more in this component of the immune system.

Once developmental processes are complete, species may invest differing amounts of nutritional resources into maintaining pools of leukocytes ready to respond to challenges by pathogens. For example, levels of some immunoglobulins are maintained at sufficiently high levels to provide immediate protection (Cohn and Langman, 1990). These so-called "natural antibodies" are only a subset of the total repertoire but are very important in determining resistance (Flajnik and Rumfelt, 2000; Boes, 2000; Cotter, 1998) and represent one of the largest components of the maintenance costs of immunity. There appears to be considerable species variability in the number of clonotypes represented in basal levels of circulating Ig, and this may represent differing levels of investment in maintaining specific immunity. Given the importance of constitutive Ig production in managing commensal microflora populations, it might be expected that species with large caeca may invest more in maintenance function.

An immune response to a pathogen requires clonal proliferation of lymphocytes, formation of germinal centers, recruitment of new phagocytes from bone marrow, and synthesis of effector molecules (e.g., lysozyme, complement). Most immune responses are also accompanied by a systemic acute phase response, which has an exceptionally high cost. Though blocking the acute phase response impairs resistance to some types of pathogens, the protective value of the acute phase response is thought to be relatively low and non-specific compared to leukocyte-mediated effector functions. Consequently, the acute phase response might be considered to have a low benefit to cost ratio, and be de-emphasized in some life-history strategies. Among chickens bred for differing rates of growth, we have detected differing investments in the acute phase response, with faster growing strains having a blunted response (Leshchinsky and Klasing, 2001). The balance between investment in adaptive immunity during development and reliance on "pay as you go" innate immunity will presumably follow predictable patterns related to life-histories. So uncovering their relationships to life-span, mode of development, dietary preferences, habitat preferences and reproductive strategy should be instructive.

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