Sex Hormones and Immunity to Infection

Sabra L. Klein • Craig W. Roberts
Editors

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Springer

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Foreword

Why Sex Matters

In the biological sciences, we tend to measure comparative differences rather than absolute values. We customarily compare treated versus untreated, exposed versus unexposed, susceptible versus resistant. Yet biologists too often neglect one of the most fundamental differences, male versus female. This distinction is clearly important; the mere fact that it has been adopted by so many species and preserved over so long a time testifies to its evolutionary advantage. By providing a powerful mechanism of diversity, sexual recombination forms a broadened substitute upon which selection pressure can act.

Surely, the careful study of sexual dimorphism will help to answer many of the questions that biologists ask. How are sex-based differences produced and how are they preserved? How do they affect survival and behavior? Most important, how can they help us to better understand astonishing and ever-changing mosaic of life on this planet?

This book proposes to dig deeply into one aspect of the question of why sex matters. Nothing has proven to be more informative than exploring the determining role that sex plays in host resistance to infection.

Among human and nonhuman animals, the prevalence (i.e., the proportion of individual infected) and intensity (i.e., severity of infection) of infection typically is higher in males than in females. Of course, this reflects differences in exposure as well as inherited differences in susceptibility to pathogens (Klein 2000, 2004; Roberts et al. 2001; Zuk and McKean 1996). Heightened susceptibility to infection is one of the leading explanations of the greater death rates among men than among women reported in several locations around the world. In general, females have more intense immune responses than males (Klein 2000, 2004; Zuk and McKean 1996). The greater immunity among females creates a double-edge sword; it is beneficial as a defense against infectious diseases, but is detrimental in the increased occurrence of autoimmune diseases (Wizemann and Pardue 2001). Sex-based differences typically become apparent after puberty (Klein 2000; Roberts et al. 2001) and several field and laboratory studies link sex differences in immune

function with circulating steroid hormones (Klein 2000, 2004; Roberts et al. 2001; Zuk and McKean 1996). Sex hormones change profoundly during pregnancy where they must modulate the immune system to facilitate a successful pregnancy among viviparous animals (Roberts et al. 1996). This task must be accomplished without increasing the general vulnerability of the mother to infection.

This book is especially timely, given the recent scientific report (Simon et al. 2005) by the Society of Women's Health Research showing that less than 3% of the funded research grants at the National Institutes of Health (NIH) in the US are awarded for the study of biological differences between males and females. This report followed the publication of an Institute of Medicine report (Wizemann and Pardue 2001) entitled *Exploring the Biological Contributions to Human Health: Does Sex Matter?* The IOM report concluded that sex differences in susceptibility, prevalence, and severity are apparent for many diseases, including cancers, heart disease, autoimmunity, and infectious diseases. These reports emphasize the need for greater research on women's health issues. Although the inclusion of women and minorities in clinical research has increased, examination of the biological differences between the sexes and how they affect health and disease has lagged (GAO 2000). By focusing on the need for including sex-based studies of infectious diseases, this book emphasizes the value of examining responses in both males and females to improve our understanding about host-pathogen interactions in both sexes. These are issues relevant to the entire scientific community.

The contributors are selected from a variety of disciplines, including microbiology, immunology, genetics, pathology, and evolutionary biology all of them have made important contributions to our knowledge of sex differences and the effects of pregnancy on susceptibility to infection. They then chronic six broad themes to represent the current trends of this diverse body of literature. The book begins with a chapter on the evolution of sex differences in susceptibility to infection. Males and females differ in the selection pressures acting on each sex; therefore, in addition to the genetic and hormonal mechanisms that underlie sex differences in immune function, evolutionary factors must also be considered (Chap. 1). Then follow two chapters dedicated to the direct effects of steroid hormones on the functioning of the immune system. The prevailing hypothesis to explain immunologic differences between the sexes is that sex hormones, in particular, testosterone, 17β-estradiol, and progesterone, influence the immune system (Chap. 2). Circulating concentrations of hormones may not be the only index of steroid hormone effects on immune function. Generally, the effects of hormones depend not only on circulating concentrations but also on the availability and affinity of target-tissue receptors. Accordingly, sex steroid hormone receptors have been identified on several classes of immune cells (Chap. 3).

As noted above, the prevalence and intensity of infectious diseases is generally higher in males than in females (Roberts et al. 2001; Simon et al. 2005; Zuk and McKean 1996). The interactions that exist between the endocrine and immune systems are important in considering why males and females differ in susceptibility to infectious agents. The editors have allotted several chapters to review evidence for sex differences in response to viruses, bacteria, and parasites, with emphasis on

the role of sex steroids (Chaps. 4–6). Chapter 7 explores the often expressed belief of "female immunological supremacy". Specifically, although males are more susceptible than females to many infectious agents, males are not more susceptible to all parasites. This phenomenon as well as the underlying mechanisms is fully addressed.

Pregnancy is a reproductive condition during which profound hormonal and immunological changes occur. How pregnancy and the associated rise in sex hormones modulate maternal immune responses and the severity of infections is discussed in Chaps. 8 and 9.

The functional significance of sex differences in immune responses to infectious agents is considered in Chaps. 10 and 11. They provide an epidemiological perspective and raise the possibility that if males and females differ in their immunological responses to pathogens, they may differ in their responses to treatments as well.

In summary, this timely volume critically reviews, in a single publication, the evolutionary origin and the functional mechanisms responsible for sexual differences in response to infection. Surely, it will become a standard reference course for those in this growing field. It brings fresh insight into the management of infectious diseases, delineates areas where knowledge is lacking, and highlights the future avenues of research. It brings us closer to an answer to the question of why sex matters.

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Sabra L. Klein
Craig W. Roberts

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Contents

Chapter 1
Sex Differences in Susceptibility to Infection: An Evolutionary Perspective

Marlene Zuk and Andrew M. Stoehr

Abstract Patterns of sex differences in parasite infection and immune responses have been noted for many decades. Although numerous explanations for such differences have been proposed, including hormonal patterns and sex-biased exposure to infective stages of pathogens, these have largely been proximate explanations that address the mechanisms immediately responsible for the findings but do not take a more integrative or ultimate approach. Here, we present an evolutionary framework for understanding the origin and maintenance of sex differences in the incidence and susceptibility to infectious disease, using life history theory and sexual selection to make predictions about when males or females in a particular species are expected to be more or less susceptible to parasites.

1.1 Introduction

Sex differences in incidence and pathogenesis of parasite infections have been of interest to parasitologists for a long time, indeed almost since the systematic study of animal parasites became established near the beginning of the twentieth century. Parasitologists examining animals collected in the field found it natural to note differences in infestations between the host sexes, and their interest was continued in laboratory experiments (Addis 1946, Solomon 1966, Alexander and Stimson 1988). Most of these studies focused on mammals, and during the

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mid-twentieth century a virtual cottage industry developed in which investigators experimentally infected laboratory rodents with identical doses of parasites and documented any resulting sex differences in the prevalence or intensity of the infection that developed (reviewed in Zuk and McKean 1998). Although exceptions could be found, the majority of research found that males were more likely to harbor parasites or to suffer more intensely from their effects than were females. Furthermore, the persistence of these patterns after experimental infestations of animals in the laboratory suggested that the sex difference was not merely due to differences in exposure to parasites, but also due to males and females behaving differently in the field and hence incurring different risks of infection.

The medical community has also known about sex differences in infectious disease susceptibility for many years. In his 1958 paper, *Biological Sex Differences with Special Reference to Disease, Resistance and Longevity*, the influential physician and medical researcher Landrum Shettles listed ways in which males suffered more from illnesses or were otherwise more fragile than women, concluding, "Females are more resistant to disease, the stress, and strain of life. In general, their biological existence is more efficient, preeminent than of males. In brief, the human male with beard and functioning testes pays the higher price."

More recently, interest and research in sex differences in parasite infections have been expanded in several ways. Firstly, researchers have extended documentation of the parasites themselves to an examination of sex differences in immune response. Here too, at least in most mammals, males tended to be more susceptible to infection, with numerous immune measures suggesting reduced responses in males (Zuk and McKean 1998). Secondly, sex differences in parasite prevalence or intensity were connected to endocrine differences, with a variety of hormones, particularly testosterone and estrogen, implicated in the observed patterns. In particular, testosterone is associated with a suppressed immune system in many mammals, although its action is likely to be mediated by other hormones (see Chaps. 2 and 3 for a much more detailed discussion of this topic). Thirdly, the role of immunity in free-living animals began to attract a great deal of attention, as scientists began to realize that susceptibility to disease was important in an ecological and evolutionary context (Sheldon and Verhulst 1996).

Finally, these observations also were seen to dovetail with another set of findings: males from a variety of mammalian species, including our own, tend to die earlier than females, regardless of the cause. A survey of 227 countries showed that women outlive men in all but a handful of places, whether their lifespan is short, as in Sierra Leone (49 years for women, nearly 44 for men), or long, as in Norway (82 years for women, 76 for men) (Kinsella and Gist 1998). The few countries where men outlive women are almost all in a state of HIV- or conflict-churned crisis, such as Zimbabwe, where women live a scant 35 years to men's 38. The gap between male and female longevity actually increases the longer that both sexes live. Kruger and Nesse compared

men's and women's mortality rates for 11 causes of death in men and women from 20 countries, including accidents and homicide as well as infectious and noninfectious diseases (Kruger and Nesse 2006). Men virtually always died earlier than women. They concluded, "Being male is now the single largest demographic risk factor for early mortality in developed countries."

Is there a common thread linking sex differences in parasite prevalence and susceptibility to the higher male mortality that results from all causes? We suggest that an evolutionary approach can unify explanations of sex differences in disease and provide a framework for the research being conducted in this area. Current thinking on the underlying theory behind the evolution of sex differences in many traits, including development of disease, is discussed below. This begins by distinguishing between proximate and ultimate explanations for such differences, as well as for other biological characteristics.

1.2 Levels of Analysis: Proximate and Ultimate Explanations in Biology

Before one can understand why sex differences in parasite susceptibility or immune responses exist, it is important to distinguish between two levels of analysis used for understanding phenomena such as "proximate" and "ultimate". Both are equally valid, but scientists often talk at cross-purposes when they conflate the two.

Proximate explanations are dissections of the mechanism behind a trait, the steps that allow the organism to behave in a particular way or exhibit a characteristic. Proximate causes occur during an individual organism's lifetime, and consist of internal developmental and physiological processes that lead, in the short term, to the phenomenon under consideration.

In contrast, ultimate explanations rely on events that occurred over evolutionary time. Understanding the selection pressures that led to the evolution of certain forms of a trait and not others can help us to understand the adaptive significance of the trait, regardless of the mechanism that makes it happen. Information about the historical sequence of events that took place over the long term, often obtained through a phylogeny of species or other taxa related to the organism in question, can sometimes yield even more information about the evolution of the trait.

Consider, for example, the question of why males of many bird species sing to attract a mate in the springtime rather than at some other time of year. A proximate explanation might invoke hormonal changes triggered by lengthening days that then alter neurochemicals in the vocal center of the bird's brain and prompt it to sing. An ultimate explanation, on the other hand, would seek the benefit that birds confining their singing to such a period would obtain. Presumably, more insects are available in the spring and summer, when the chicks require feeding by their

parents, than at other times of year. Individuals that sing, and breed, in the spring are thus more likely to successfully rear their offspring and pass on the genes associated with their responsiveness to the increasing hours of daylight. Both explanations are valid and important to a full understanding of the problem, but they operate at different levels of analysis. Some refer to proximate-level questions as "how" questions and ultimate-level questions as "why" questions, but we think they can both be placed in either format and do not see such a dichotomy as particularly helpful.

With respect to sex differences in susceptibility to parasites, explanations about different hormone levels or the differential exposure of the sexes to the infectious stages of parasites are all proximate explanations. Understanding the interactions among, for instance, testosterone, estrogen, or corticosteroids, and various immune system parameters is important in deciphering the mechanism behind observations or experimental demonstrations of such sex differences, but it does not speak to the selective forces that produced these interactions in the first place. For that, an ultimate explanation is required. Furthermore, focusing at an ultimate level of analysis helps to put "exceptions to the rule" in perspective. If females of a particular species happen to be more susceptible to parasites than are males (as discussed in Chap. 7 of this book), while most other species in the group show the opposite pattern, we can attempt to understand how natural and sexual selection in that species might have produced such a contrary pattern. Discovering that testosterone is not always associated with a suppressed immune system, thus, does not negate the ultimate explanation that males are generally expected to be more susceptible to parasites, though it might call into question the mechanism behind the observation.

1.3 Sexual Selection and Sex Differences in Infection

What, then, is an appropriate framework for addressing the ultimate explanation for sex differences in infection? Here, we briefly review sexual selection theory and current thinking on the evolution of reproductive strategies.

Sexual selection is the counterpart to natural selection, and refers to the differential reproduction of individuals due to competition over mates, as opposed to differential reproduction due to the ability to survive. Like natural selection, sexual selection was originated by Charles Darwin, who distinguished between traits used for survival and those used in acquiring mates. He devoted an entire book, published in 1871, *The Descent of Man and Selection in Relation to Sex*, to the latter. He pointed out that many apparently unusual-appearing traits are actually used in daily life, like the long curved bill on a bird, for example, which may help in feeding. But certain other traits are not so clearly functional, and they are frequently confined to one sex. In some birds of paradise, for instance, the male has a pair of ornamental feathers so long they actually impede his flying ability. Traits such as these are common in the animal kingdom, and include vocal signals like bird and frog song as well as visual signals like elaborate plumage or displays.

Darwin further noted that traits occurring in only one sex could be of two types. First are the primary sexual characters, the basic morphology such as the gonads that enable males to produce sperm and females to produce and nurture eggs. The evolution of these traits is fairly obvious, and requires little special explanation. Other traits, such as the bright colors of many birds or the structures like antlers on male deer, were not so simply understood. Darwin called such traits as secondary sexual characters, and in many cases they are actually detrimental to survival, via an enhanced conspicuousness to predators or other natural enemies or via the energetic cost of producing them.

Darwin proposed that secondary sexual characters could evolve in one of two ways. First, they could be useful to one sex, usually males, in fighting for access to members of the other sex. Hence, the antlers and horns on male ungulates or beetles of some species. These are weapons, and they are advantageous because better fighters get more mates and have more offspring. The second way was more problematic. Darwin noted that females often pay attention to traits like long tails and elaborate plumage during courtship, and he concluded that the traits evolved because the females preferred them. Peahens, thus, were expected to find peacocks with long tails more attractive than those with shorter tails. The sexual selection process, then, consisted of two components: male–male competition, which results in weapons, and female choice, which results in ornaments.

Although the scientific community did not accept sexual selection as readily as natural selection, the theory was finally embraced by the middle of the twentieth century, and research into the evolution of sex differences accelerated. Rather than assuming that females would always be the choosy sex and males the competitive one, however, scientists focused on the ways in which each sex is limited in achieving higher reproductive success.

Evolutionary biologist Robert Trivers (1972) pointed out that females and males usually inherently differ because of how they put resources and effort into the next generation, which he termed parental investment. Female reproductive success is limited by the number of offspring a female can successfully produce and rear. Because they are the sex that supplies the nutrient-rich egg, and often the sex that cares for the young, females will usually leave the most genes in the next generation by having the highest quality young they can; the upper limit to the quantity is usually rather low. Which male they mate with could be very important, because a mistake in the form of poor genes or no help with the young could mean that they have lost their whole breeding effort for an entire year. Ornaments could evolve as indicators of this high quality. Males, on the other hand, can leave the most genes in the next generation by fertilizing as many females as possible. Because each mating requires relatively little investment from him, a male who mates with many females sires many more young than a male mating with only one female.

Variance in male reproductive success is thus expected to be higher, on average, than variance in female reproductive success, which in turn selects for what might be termed a "live hard, die young" overall strategy for males, at least with respect to mating behavior. In elephant seals, for example, a single male may sire more than 90% of the pups in a colony, leaving the vast majority of males with no offspring,

while females will virtually always give birth to a single pup. Males battle ferociously among themselves for dominance on the breeding grounds.

With regard to susceptibility to infection, these sex differences in reproductive strategy may provide the ultimate selective force behind increased male vulnerability to infections. If males require, for example, testosterone for aggressive behavior and the development of male secondary sexual characters, selection for winning at the high-stake game that the males play may override the cost in terms of any immunosuppressive effects of the hormone. Sex differences in infection may, thus, simply reflect the larger pattern of differential selection on the sexes.

1.4 The Role of Life History Theory

Testosterone alone, however, is not the sole means by which males and females differ in their physiology. A more general approach to the question of which sex is expected to have evolved greater disease susceptibility comes from life history theory, which examines the evolution of such life "decisions" as how many offspring a species is expected to reproduce and how large those offspring should be at birth or hatching. The underlying assumption is that organisms have a finite pool of energy or resources to draw from, and therefore must allocate that energy to different tasks. Because the resources used for one function are unavailable to another, trade-offs between traits such as growth rate and body size, or between the size and number of offspring, are expected. Life history theory explains many of the apparently maladaptive features of life; animals cannot be good at everything. Along these lines, despite the obvious advantage of being resistant to disease, susceptibility is of course rampant. As with other life history traits, it has seemed logical to conclude that resistance is traded off against the need for investment in other important characters, such as competitive ability or development time (Roff 1992). We assume that animals remain vulnerable to pathogens because being resistant is costly. Evolution has, therefore, not perfected the ability to fend off parasites – i.e., produced organisms that are completely parasite-free – because for most if not all individuals, resources are better expended on other physiological activities or processes.

This view of an animal's reaction to infection as simply another drain on a limited pool of resources provides another kind of ultimate explanation for sex differences in susceptibility to parasites. Combined with sexual selection theory it means that we can begin to ask why we see the patterns that we do, not from the standpoint of an individual species' quirks of immunology, but by examining the way natural and sexual selection are expected to act on life history, including disease resistance.

1.5 Empirical Approaches

One of the earliest discussions of sex differences in disease outcome, from an evolutionary-theoretical perspective was that of Zuk (1990), who emphasized the

inherently different means by which males and females maximize reproductive success in many species. In those species where male fitness is heavily dependent upon maximizing mating success (i.e., polygynous species, in which a single male may mate with multiple females), males may benefit from sacrificing immune defense if those resources can, instead, be devoted towards mating efforts. In monogamous species, males typically maximize fitness by assisting in the rearing of offspring, as do the females. Thus, this hypothesis predicts that in monogamous species, males and females will have similarly effective immune defenses, but as the mating system departs further from monogamy towards polygyny (meaning that the strength of sexual selection on males increases), the sex differences in immune defenses, with males showing the less effective defenses, increase (Zuk 1990). Since Zuk (1990), this basic hypothesis and associated predictions have been developed in several other papers (Zuk and McKean 1996; Rolff 2002; Zuk and Stoehr 2002). One of the strengths of this hypothesis, as an "ultimate explanation," is that the predictions apply to taxa other than mammals, including those, such as insects, that lack the hormone testosterone.

A proper test of the hypothesis' primary prediction requires sufficient knowledge of (and variation in) both mating system (or some measure of the strength of sexual selection) and immune defense in a number of species-data that are lacking for many systems, although increasing all the time. Measures of parasitic infections, such as prevalence (proportion of hosts infected) or intensity (number of parasites per host) are typically easier to acquire than more direct measures of immune defense. Nevertheless, the available data on infection levels do highlight interesting patterns, and, not surprisingly, raise more questions. A study examining infection levels across arthropods found no consistent evidence for sex biases in infection prevalence or intensity (Sheridan et al 2000). However, a consistent pattern was lacking not because there were no host taxa for which males were more heavily parasitized, but rather because there were similar numbers of taxa in which females were more heavily parasitized.

Even in vertebrates, where we might expect consistent male-biased infection with parasites because of the immunosuppressive effects of testosterone, things are not so simple. For example, Poulin (1996) found evidence for male-biased parasitic infections in birds when the prevalence of helminth infections was considered, but not when the intensity of infection was considered. McCurdy et al. (1998) found no evidence for an overall sex bias in parasitic infections, but when considered by parasite taxon, the prevalence of *Haemoproteus* infections was female-, not male-biased; this was true even in polygynous species, where the male-biased infections would be most expected. Moore and Wilson (2002) examined the relationship between sexual selection and parasitic infection across mammals. Using methods that controlled for correlations between traits due to shared ancestry, Moore and Wilson (2002) used two measures of the strength of sexual selection – mating system and sexual size dimorphism – to determine if sexual selection was associated with sex differences in infection with parasites. As predicted, increases in polygyny or greater male size were associated with greater sex differences in parasitic infection. One of the most interesting findings of the study was

that in those species where females are the larger sex, parasitic infection was female-biased (i.e., females had more parasites). However, in these species, larger female size is not thought to be due to sexual selection on females – thus, the cause and effect relationships among sexual selection, sex differences in parasitic infection, and body size appear complex indeed.

To the best of our knowledge, no large comparative (i.e., multiple species, phylogenetic controls, and sexual selection measures) study utilizing more direct measures of immune defense to address sex differences in immune defenses, rather than parasites themselves, has been conducted. However, an alternative and increasingly popular approach to empirically testing the hypothesis that sexual selection influences sex differences in immune defenses is to experimentally manipulate, in a single species, factors such as the strength of sexual selection, mating history and resource abundance. These studies, too, are revealing that the relationship between sexual selection and immune defense is complex. Indeed, in both invertebrates and vertebrates, the direction or presence of sex differences in immune function may depend upon not only the factors manipulated in the experiment, but also which component(s) of immunity were assessed (Klein 2000; Adamo et al. 2001; Hosken 2001; Fedorka et al. 2005; McGraw and Ardia 2005; McKean and Nunney 2005; Rolff et al. 2005; McKean and Nunney 2008). For example, in crickets, sex differences with phenoloxidase activity, one measure of potential immune defense, were apparent in later stages, but not in earlier stages of development. However, no sex differences were found at any stage for hemocyte number (a count of one of the cell types involved in arthropod immune defense) (Adamo et al. 2001).

1.6 Theoretical Approaches

Given these complex patterns, what are we to make of the underlying evolutionary, i.e., ultimate, reasons for sex differences in immune defense? Were the original formulations of the hypothesis, such as those by Zuk (1990) or Rolff (2002) incorrect? Here, we briefly discuss some of the more recent theoretical investigations into the problem of how sex differences in immune defense might have evolved.

All models, verbal or quantitative, make assumptions. Often, these assumptions are less than obvious; this is particularly true in the case of verbal models. The model as articulated by Zuk 1990, Rolff 2002, and others makes two assumptions that may be important for understanding variation in the magnitude and direction of sex differences in susceptibility to parasitic infection. The first assumption is that female fitness is more dependent upon longevity than is male fitness. The second assumption, which is probably the more important of the two, is that the most important benefit of immunocompetence is to increase survival, or, if one likes, that the primary cost of parasitic infection is death. From the perspective of a resource allocation problem, the model with these assumptions in place can be represented graphically, as in Fig. 1.1a. It is clear that with these assumptions in place, the sex
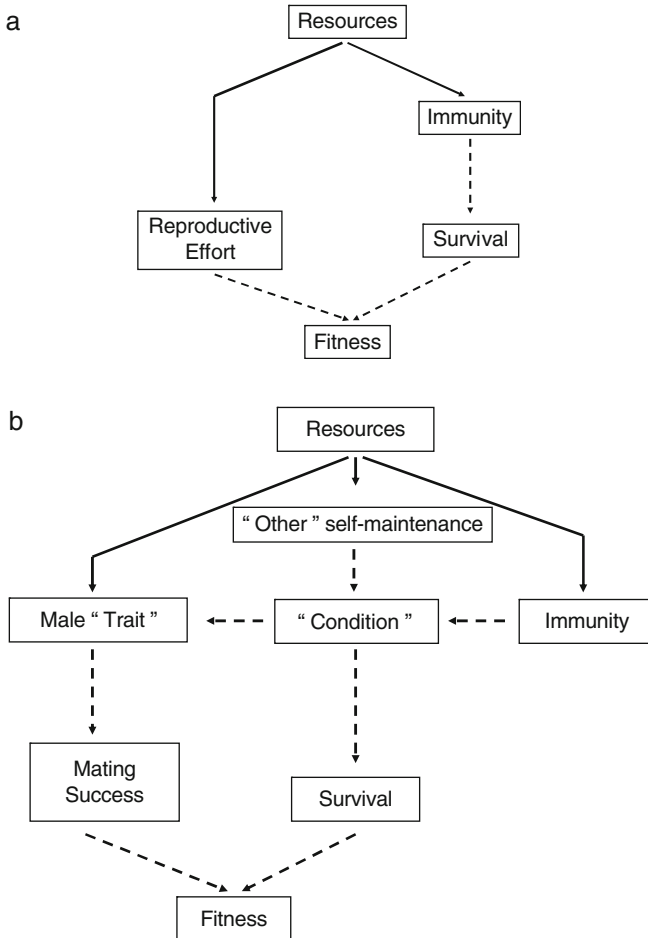
Fig. 1.1 (**a**) Resource allocation to immunity and reproductive effort, assuming that the benefits of immunity only affect survival. In this case, it is clear that the sex that invests the most in survival must necessarily invest more in immunity (solid arrows represent resource allocations; dashed arrows are causal relationships) (**b**) Resource allocation when immunity can affect both survival and mating effort, due to the benefits of immunity for "condition". Shown here is the male case; in females, reproductive effort is simply fecundity

that values survival (typically argued to be females) will be the sex that invests in immune defense.

However, it is not clear how broadly these assumptions apply. For polygynous mammals, it appears that, indeed, longevity is more important for male fitness than for female fitness. But long-term studies in several bird species show that longevity accounts for approximately 60% of the variation in fitness for both females and males, and ranges from about 30 to 80% for both sexes

(summarized in Newton, 1989). Longevity may account for a considerable proportion of variation in fitness for both sexes in many insects, as well (Clutton-Brock 1988).

Even in species where longevity is of less importance to males than to females, should we always expect males to invest less in immune defense? Parasites may kill their male hosts, but many infections may reduce the general health or condition of their hosts, which, in turn, may affect traits important for mating success such as bright coloration or energetically expensive courtship behavior, without being lethal. It could be argued such a cost of parasitic infection could be even more detrimental to males than to females, because while a sublethal infection may reduce female fecundity, it may not necessarily prevent her from being mated and rearing some offspring. In some mating systems, however, a parasitized, unhealthy (and therefore less attractive) male may have zero fitness. Thus, the (second) assumption that the primary cost of parasite infection is death, and its implicit accompanying assumption – that the sublethal effects of parasitic infection (e.g., development of disease) are the same for each sex – may not always be true. (This is addressed later – see the reference Blanco et al. 2001 and Tseng 2004)

Stoehr and Kokko (2006) examined the importance of these assumptions by constructing a model of resource allocation to various fitness components, including disease resistance, that would not only allow survival to play an important role in the fitness of both sexes, but more importantly, acknowledge that parasites have sublethal effects, and that these may not be the same for the sexes. In addition, the model incorporates these ideas by also allowing the effects of parasitic infection (and therefore the benefits of immunity) to be realized through the effects of "condition," on the traits that are important to fitness. For the purposes of the model, condition can be defined as that attribute of an organism that is not only affected by resource allocation to it, but also in turn affects other traits such as survival and fecundity; that is, in this model "condition" is what we might generally refer to as the "health" of the organism.

The graphical representation of this model is shown for males in Fig. 1.1b. (The female case is basically the same, except that instead of the male trait and mating success, these are collapsed into female reproductive effort, or fecundity). In the model, resources are allocated to immune defense, reproductive effort (e.g., a male's extravagant plumage or courtship song), and other forms of basic self-maintenance. Immunity, along with other forms of self-maintenance, has positive effects on "condition," and condition in turn has positive effects on survival and on male reproductive effort (i.e., the male "trait"). In this scenario, immunity does have costs, in that immunity and male reproduction compete for limited resources. However, we do not necessarily expect males to simply maximize fitness by investing all resources into reproductive effort, because if immunity is sacrificed entirely, condition, and therefore both survival and reproductive effort, are compromised (the mathematical details of the model, which are explained in Stoehr and Kokko (2006), insure that if no resources are invested in immune defense, then condition, and therefore survival, is zero). Thus, this formulation more realistically represents what we know to be the more general effects of

resistance to infection on survival and reproductive effort – i.e., it does not assume that immune defense only evolved in the context of increasing survival.

Stoehr and Kokko (2006) then explored the implications of this model by first constructing a series of mathematical equations that expressed the relationships between these different components of the model and allowed these relationships to take varying shapes. Of primary interest to us for understanding sex differences in immune function are three particular relationships. One is the relationship between the male "trait" and his mating success; this is a measure of the strength of sexual selection. Also of interest is the relationship between immunity and condition. While this could reflect details of the immune system, in the model of Stoehr and Kokko (2006) this is constructed more generally and can be thought of as the impact of parasites and disease outcome on condition. In this manner, it incorporates not only details of immune defense but also variation in parasite combinations, parasite virulence, and behavior that leads to differences in host exposure to parasites, etc. Such a broad approach is important, because the impact of parasites may differ between the sexes; for example, males may be exposed to more (or fewer) parasites because of their courtship behaviors (Tinsley 1989, Zuk and Kolluru 1998). Finally, there is the relationship between condition and reproductive effort. This is, for males, the condition-dependence of traits such as bright coloration, elaborate courtship dances, or loud or complex calls and dances: males in better condition produce more vigorous displays. For females, this is the condition-dependence of fecundity: females in better condition produce more or healthier offspring. Given how different the forms of reproductive effort take for males and females, it would seem highly unlikely that condition would have identical effects on reproductive effort for both sexes. Thus, by varying the shapes of the relationships between immune defense and condition, and condition and reproductive effort, the potential importance of the assumption that the nonlethal effects of parasites are similar (and negligible) for the sexes can be assessed.

Stoehr and Kokko (2006) examined these assumptions numerically, through an evolutionarily stable strategy (ESS) approach. An evolutionarily stable strategy is one that would persist in a population even if a mutant form pursuing an alternative strategy were to enter the population. Stoehr and Kokko (2006) began with an arbitrary resource allocation strategy for a population, given certain parameter values for the strength of sexual selection, the impact of parasites on condition, and the condition-dependence of reproductive effort. Then new resource allocation strategies were explored, and any that resulted in higher fitness could "invade" and replace the old strategy; when the best strategy to adopt is the existing strategy, the evolutionarily stable (i.e., "best") strategy has been achieved.

Recall that the primary prediction of the hypothesis for sexual dimorphism of immune defense is that as the strength of sexual selection increases, the magnitude of the difference between sexes, with males showing an inferior immune response, is expected to increase. Stoehr and Kokko (2006) found that, indeed, this prediction is supported provided that (a) the impact of parasites on condition is the same for the sexes; (b) the condition-dependence of reproductive effort is the same for the sexes; and (c) neither of these effects is particularly strong. If instead parasites are

highly detrimental to condition and/or reproductive effort is highly dependent on condition, then males cannot afford to sacrifice immune defense to improve mating success, even in the face of very strong sexual selection. As a result, both sexes invest in immune defense equally. More importantly, the model shows that if the impact of parasites on condition is greater for males than for females, males should invest more of their resources into immune defense than should females, even in the face of strong sexual selection (Fig. 1.2). A similar, though not quite as dramatic, effect is found if male reproductive effort is more condition-dependent than is female reproductive effort. In other words, even if the effects of sexual selection are to diminish male investment in immunity below that which would occur in the absence of sexual selection altogether, this diminishment may still not be sufficient to cause males to invest less in immunity than do females (Fig. 1.2; *upper thin solid line*).
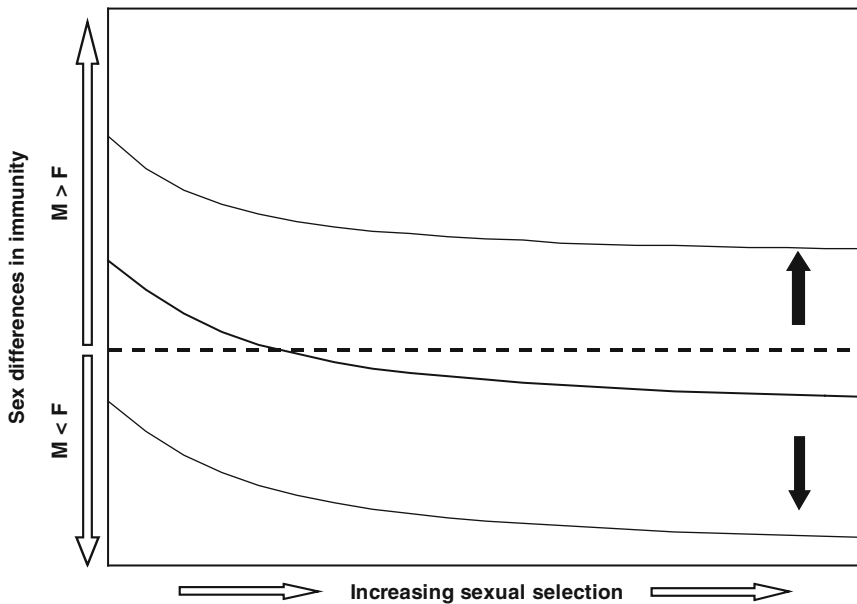

Fig. 1.2 Sex differences in immunity as a function of sexual selection. The thick solid line represents the case when the condition-dependence of reproduction and the effect of immunity on condition are equal for the sexes; when sexual selection is absent or weak males should invest more in immune defense than should females (i.e., thick, solid black line is above the dashed line, in the region of M>F investment in immunity). As the strength of sexual selection increases, the female bias in investment in immunity increases. However, if parasites have particularly strong negative effects on condition in males, and/or if male reproductive success is highly dependent on condition, relative to those same effects in females, males should invest more in immunity than should females, even when sexual selection is strong (thin solid line raised above the thick solid line, and never crossing dashed line). Of course, the converse situation may mean that males never invest more in immunity than do females (lower thin solid line)

The results of the simulation by Stoehr and Kokko (2006) suggest that the validity of the assumptions implicit in the verbal models arguing for inferior male immune defenses when sexual selection is strong may be very important. We not only know that in many cases male secondary sexual traits are condition-dependent, but, in fact, theory suggests that we should expect these traits to be condition-dependent (Andersson 1994). Of course, we also expect female fecundity to be condition-dependent, so the question, for our purposes, becomes "When do we expect male fitness to be more condition-dependent than female fitness?" because these are the cases where we might (given certain other assumptions) expect males to invest more in their immune than do females. Unfortunately, as any biologist who has ever tried to quantify (or even define!) condition will realize immediately, comparing condition and condition-dependence between the sexes is hardly trivial. It would not simply be enough to examine the correlation between some measure of condition and secondary sexual trait (for males) and fecundity (for females) because ultimately, we would also need to know something about how that male secondary sex trait expression translates into fitness. However, there may be some well-studied systems where such a comparison might be possible.

Perhaps, a slightly more tractable question is whether similar parasitic infections affect the condition of the sexes equally. This question is not free from the inherent difficulties of measuring condition, but there is at least some evidence to suggest that, when such a comparison can be made, the answer is that parasites do not always have the same effects on male and female condition (Blanco et al. 2001; Tseng 2004). For example, in magpies, there is a negative correlation between lice infestation and nutritional condition (in this case, body mass adjusted for skeletal size) in both sexes, but the relationship is stronger for males (Blanco et al. 2001). And in mosquitoes, infection with parasites reduces male body size more than it does female body size when the mosquito larvae are reared at high density, but at low larval densities, parasites have a greater impact on female body size (Tseng 2004). Furthermore, because the model of Stoehr and Kokko (2006) includes potential exposure differences as part of "parasitic impact," behaviors that bias exposure in one sex may also be important, and such behaviors have been found (Tinsley 1989; Zuk and Kolluru 1998; Riemchen and Nosil 2001). Finally, it must be remembered that these two important effects – i.e., the impact of parasites on condition and the condition-dependence of reproductive effort – may interact in concert, to increase the magnitude of sex differences in immunity, or in opposition, to diminish or erase sex differences in immunity.

Like all models, Stoehr and Kokko's (2006) make its own assumptions and has its own limitations. The primary purpose of this model was to examine the logic of the basic arguments (or, put another way, the importance of the implicit assumptions) put forth in earlier less quantitative treatments of the sexual selection versus male immune defense hypothesis. As such, the model is successful as it reveals that these assumptions may be crucial in understanding how sexual selection and immune defense interact to produce or eliminate sexual dimorphism in immune defense. However, it is not a detailed model of immune defense. For example, Stoehr and Kokko (2006) ignore potentially important factors such as the complex

and multifaceted nature of immune defenses, host–parasite coevolution, and the genetics of resistance. In addition, the model ignores the possibility that individuals (or the sexes) may differ in the amount of resources they acquire.

Although it seems unlikely that incorporating any of these factors will reveal that things are more simple than they appear, these are certainly factors that should be incorporated, in as much as is possible, in future theoretical and empirical approaches to understanding sexual dimorphism in immune function. Indeed, several recent models addressing optimal allocation of resources to immune defense raise several interesting points. None of these models addressed sex differences in immunity, but their findings should be incorporated into future theoretical treatments of this problem. For example, one of the underlying assumptions of earlier treatments of sex differences in susceptibility to infection and the manifestation of disease was that females would invest more in immune defense because they are often the longer-lived sex; that is, it was assumed that inherently long-lived organisms would favor immune defense greater than short-lived organisms. This assumption is challenged in models by van Boven and Weissing (2004) and Miller et al. (2007). Both of these studies found that, under some conditions, optimal investment in immune defense is maximal at intermediate lifespans, not at the longest lifespans. One of the reasons this appears to be so is because of demographic processes: long-lived species do not have high demographic turnover, and therefore do not supply the "fuel," i.e., susceptible individuals, necessary to support some species of parasites (van Boven and Weissing 2004; Miller et al. 2007). As a result, there is less benefit to investing in costly immune defenses in these species. Not surprisingly, however, these conclusions depended on certain assumptions as well; for example, if immunity was innate, instead of acquired, then optimal investment increased with lifespan (Miller et al. 2007).

As mentioned above, Stoehr and Kokko's (2006) model did not consider that males and females might start with differently sized resource pools. Sex differences in resource acquisition might occur, however, if one sex is forced, to a greater degree than the other, to sacrifice, say, foraging effort in order to invest in reproduction. In a model of optimal resource allocation to immune defense, Medley (2002) found that optimal allocation of resources to immune defense calls for little to no allocation in starved individuals, peaks in those individuals with intermediate levels of resources, but then falls again in "well-fed" individuals. Hosts with more resources, i.e., "in better condition," may be better able to tolerate some level of infection, such that the relationship between parasite loads and condition or "quality" may be complex (Medley 2002). A similar problem was addressed by Houston et al. (2007), who modeled optimal allocation of efforts to foraging versus immune defense. In addition, Houston et al. (2007) show that whether individuals of a given state invest primarily in foraging or immune defense is not simply a matter of current nutritional state, but of environmental predictability. In more stable environments, food availability and allocation to immune defense tend to be positively related, but as the environment becomes more unpredictable, this relationship no longer holds.

1.7 Future Directions

Comparative studies of parasite infections in many different kinds of animals, as well as experimental studies of immune defense in single species and theoretical explorations of the role of resource allocation in the evolution of immunity, all suggest that it is simplistic to expect one sex to routinely have an inferior immune ability, even in species in which sexual selection has been intense. The original hypothesis that males were likely to have evolved a greater susceptibility to parasites was on the right track, in that it identified a useful way of thinking about the evolution of such sex differences. A more general perspective on the problem of resource allocation to defense against parasites as well as other outlets should prove even more valuable. The collective findings, both empirical and theoretical, clearly support the idea that life history differences between the sexes matter in understanding sex differences in disease, and that these differences can be most profitably understood in an evolutionary framework. The challenge now is to understand exactly how the differences matter; when we understand the details and mechanisms, we will be able to see why sex differences in immunity are sometimes male-biased and at other times female-biased.

To achieve this understanding, we suggest that a number of issues should be addressed. More large-scale comparative studies, conducted in a phylogenetic context, which examine immunity across species in a variety of taxa to uncover important correlates of sex differences in immunity, will be invaluable. These types of studies can reveal broad, consistent patterns and identify potentially important causal factors that can then be addressed experimentally. However, note that the evidence to date suggests that sex differences in immunity are dynamic, and may change over the course of the life history of an organism, due to changes in external factors such as resource abundance, and may vary with different components of immune defense or different parasites. For example, in *Drosophila melanogaster*, female larvae are more resistant than male larvae to a larval parasite, there are no sex differences in resistance to a pupal parasite, whereas in adult flies, there are sex differences in resistance to a microsporidian, but not to a fungal, infection (Kraaijeveld et al. 2008). Furthermore, sex differences in resistance to bacterial infection in adult *Drosophila* are highly labile: sexual activity reduces male but not female resistance, whereas resource deprivation reduces female but not male resistance, resulting in variation in the direction of sex differences in immunity depending upon how these factors are manipulated (McKean and Nunney 2005).

A relatively unexplored but potentially fruitful area of research is the intersection between population dynamics and sex differences in parasite resistance. For example, in free-living yellow-necked mice, antihelminthic treatment of a dominant parasitic helminth in males reduces infections in females in the population as well, but removal of the same parasite from females has no effect on infections in males (Ferrari et al. 2004). There is also ample evidence from a variety of species that immunity varies seasonally (Nelson and Demas 1996; Altizer et al. 2006; Martin et al. 2008). In the future, we hope to see these kinds of ecological factors

considered alongside the life history perspective we have outlined here, and these, in turn, combined with approaches that consider the multifaceted nature of the immune system (Lee 2006). The result should be a much greater, integrative understanding of sex differences in immunity than could be achieved by any single approach alone.

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Chapter 2
Effects of Sex Steroids on Innate and Adaptive Immunity

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Abstract Estrogens and androgens are classically recognized as reproductive sex steroid hormones because of their well-documented effects on reproductive tissues. However, extensive research in diverse biological disciplines have clearly established that reproductive hormones have broad physiological effects on nonreproductive tissues, including the immune, central nervous, cardiovascular, and skeletal systems. Thus, the term "sex/reproductive hormones" describes only a narrow (albeit important) aspect of biological effects of sex steroids. In this chapter, the effects of sex hormones on the innate and adaptive immune system are highlighted. Generally, estrogens upregulate proinflammatory cytokines (e.g., IFNγ) and IFNγ-inducible molecules (nitric oxide, NOS2, and COX2), whereas androgens suppress proinflammatory responses. Immunomodulation by sex steroids may have both physiological and pathological implications (e.g., sex differences in immune capabilities and in inflammatory diseases, respectively).

2.1 Sources of Sex Steroids: Physiological and Exogenous

Estrogens are produced in gonadal and extra-gonadal tissues. 17β-estradiol, is principally produced by theca and granulosa cells in the ovaries of premenopausal women (Simpson 2003). In theca cells, androstenedione is converted into testosterone by aromatase. Testosterone and androstenedione are then taken up by granulosa

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cells and converted by the aromatase enzyme to 17β-estradiol in premenopausal women (Williams et al. 1998). The levels of estrogen in women vary physiologically during menstrual cycle stages, pregnancy, and with age. In premenopausal women, the physiological range of estrogen during the menstrual cycle is between 40 and 400 pg ml^{-1} (Ruggiero and Likis 2002). Estrogen levels are markedly increased during pregnancy. In the postmenopausal stage, estrogen levels drop significantly (Akhmedkhanov et al. 2001). In mice, the physiological levels of 17β-estradiol found in the serum are as follows: diestrus 20–30 pg ml^{-1}, estrus 100–200 pg ml^{-1}, and during pregnancy 5,000–10,000 pg ml^{-1} (Bebo et al. 2001). Extragonadal estrogen synthesis occurs in mesenchymal cells of adipose tissue, breast, osteoblasts and chondrocytes of bone, vascular endothelium, aortic smooth muscle cells, and several sites in the brain (Simpson 2003). These sites are important sources of estrogen in men and postmenopausal women. Estrogens produced at these sites, unlike that secreted by ovaries, tend to act locally at high concentrations (Labrie et al. 1998).

In addition to natural estrogens, synthetic estrogens (e.g., 17α-ethinyl estradiol) are extensively prescribed as oral contraceptives to premenopausal women and as estrogen replacement therapy for postmenopausal women (Yin et al. 2002). Additionally, a third category of estrogenic compounds, referred to as environmental estrogens, is now recognized. Environmental estrogens account for a large component of endocrine disrupting chemicals (EDC). A number of EDC, including estrogens also have been shown to affect the immune system (Ansar Ahmed 2000). These estrogens can mimic or block natural hormones. Due to their ability to accumulate in adipose tissue and the fact that they are biologically active at very low concentrations, EDCs can accumulate and act cumulatively to alter the immune and reproductive systems (Soto et al. 1995). In this chapter, we detail the effects of estrogens (in particular, 17β-estradiol or E2) and androgens (in particular, testosterone) on the immune system; progesterone, another sex steroid that has profound effects on the functioning of immune cells, is discussed in detail in Chaps. 9 and 10 pertaining to pregnancy.

Testosterone is the principal androgen secreted from Leydig cells in testes of males and in small quantities from theca cells in ovaries of females. Importantly, testosterone is essential not only in sexual development and other reproductive processes but also for modulating immune responses. Males are, in general, more prone to infectious diseases, both in terms of prevalence and intensity, over females partly because of the suppressive effects of testosterone and its metabolite dihydrotestosterone (DHT) on the immune system (Choudhry et al. 2006; Easterbrook et al. 2007; Schuurs and Verheul 1990), Castration has beneficial effects on the immune system following trauma and hemorrhage (Yokoyama et al. 2002). Consistent correlations have been observed between endogenous testosterone levels and the burden of parasites, such as *Babesia microti* and *Plasmodium vivax* (Barnard et al. 1996; Muehlenbein et al. 2005), which is detailed in Chap. 6 of this book. In addition to affecting responses to parasites, androgens affect the development of the immune system as castration results in increased thymus size in mice (Olsen et al. 1991).

2.2 Sex Steroid Regulation of Innate Immune Cells

Antigen invasion into the body is largely prevented by physical barriers that act as a first line of defense. These physical barriers (such as skin, mucosal tissue of gastrointestinal, respiratory, reproductive, and urogenital tracts) are usually fortified by chemical barriers (e.g., mucus, saliva, and tears which contain protease enzymes). Cellular innate immune cells such as neutrophils, macrophages, natural killer (NK) cells, and dendritic cells (DCs) act as the second line of defense. The nature of this response enables effector cells to recognize a number of molecules widely expressed by groups of microbes and to clear or curtail their multiplication by various mechanisms such as phagocytosis and lysis of infected cells. Innate immune cells, predominantly macrophages and DCs, produce cytokines that aid in the activation and influence the nature of the adaptive immune system.

2.2.1 Neutrophils

Neutrophils or polymorphonuclear cells (PMNs) are the "first cellular responders" to counter antigenic invasion. These cells kill pathogens by two complementary effective mechanisms (1) phagocytosis, and (2) release of potent toxic oxygen-free radicals generated by a respiratory burst. Neutrophils contain a population of primary and secondary granules. Primary granules are composed of enzymes, including myeloperoxidase, acid hydrolases (i.e., cathepsins), lysozymes, and neutral proteases. Secondary granules consist of lactoferrin, lysozymes, and collagenases. Neutrophils migrate to sites of injury or inflammation in response to chemoattractants released by damaged tissues where they trap antigen in a vacuole called a phagosome. Phagosomes fuse with primary granules to form phagolysosomes (Faurschou and Borregaard 2003). The phagolysosome is a hostile environment capable of destroying many, but not all, pathogens. Concurrently, neutrophils aid in destroying microbial pathogens by another mechanism called respiratory burst by sequential conversion of oxygen to toxic superoxide anion, hydrogen peroxide, and hypochloride ion in the presence of NADPH oxidase, superoxide dismutase, and myeloperoxidase, respectively (Hampton et al. 1998).

Estrogen regulates both the number and function of neutrophils. For example, estrogen has been shown to suppress bone marrow production of leukocytes including PMNs (Josefsson et al. 1992; Wessendorf et al. 1998). This possibly is in part due to estrogen effects on the bone (osteopetrosis), which tends to occlude the bone marrow cavity. 17β-estradiol, ethinyl estradiol, and idoxifene, a selective estrogen receptor modulator (SERM), but not 17α-estradiol, significantly reduce neutrophil chemotaxis (Delyani et al. 1996; Ito et al. 1995) as well as adherence to the vascular endothelium (Delyani et al. 1996). Although 17β-estradiol prevents neutrophil infiltration and organ damage following trauma-hemorrhage, the mechanism by which it inhibits neutrophil transmigration remains unknown. Estrogens

can alter neutrophil chemotaxis and function by modulating the release of chemoattractants such as CXCL8 from monocytes (Pioli et al. 2007), and CXCL8, CXCL10, CCL5 from keratinocytes (Kanda and Watanabe 2005). Furthermore, estrogens decrease chemotaxis of neutrophils by altering the expression of adhesive proteins, such as intracellular adhesion molecule-1 (ICAM-1) and therefore, protect against myocardial ischemia-reperfusion injury and myeloperoxidase activity (Squadrito et al. 1997).

17β-estradiol and weak estrogenic analogs (e.g., estrone and estriol) significantly reduce neutrophil function as indicated by decreased superoxide anion production (Abrahams et al. 2003; Bekesi et al. 2007). Interestingly, neutrophils in females have increased resistance to activation by burn or trauma hemorrhage compared with those in males (Deitch et al. 2006). 17β-estradiol is capable of limiting neutrophil activation, as reflected by decreased CD11b expression and respiratory burst activity in response to trauma-hemorrhagic shock or burn injury (Deitch et al. 2006). The salutary effects of estrogen on attenuation of inflammatory responses are mediated by decreased neutrophil infiltration at sites of injury/inflammation, improved injury markers (e.g., myleoperoxidase activity), decreased cytokine production (e.g., TNF-α, IL-6, and IL-1β), reduced chemokine levels (e.g., cytokine induced neutrophil chemo-attractants (CINC1, CINC2, and CINC3), reduced monocyte chemoattractant protein-1 (MCP1 or CCL2)) and decreased expression of inflammatory mediators (e.g., P-selectin and intercellular adhesion molecule (ICAM1)) (Cuzzocrea et al. 2008; Hsu et al. 2007; Yu et al. 2007).

Despite the fact that estrogens can affect neutrophil-mediated immune responses, some reports suggest that estrogen does not reduce neutrophil infiltration into cardiac muscle (Cavasin et al. 2006; Tiidus et al. 2002), myeloperoxidase activity (Tiidus et al. 2002), or neutrophil degranulation and oxidation (Cave et al. 2007; Chiang et al. 2004). However, during endometriosis, an estrogen-dependent autoimmune disorder affecting women of reproductive age, estrogen enhances the responsiveness of cells to IL-1β, which acts directly to upregulate CXCL8 (i.e., IL-8), a chemokine involved in active angiogenesis and recruitment of neutrophils (Akoum et al. 2001). This suggests that estrogen may not attenuate inflammation in all cases, and a number of variables such as the dose of estrogen, tissue, and type of injury/inflammation may influence the immunomodulatory effect of estrogen.

2.2.2 Macrophages

The term macrophage is derived from the Greek words: macros: large, great, and phagein: eat; "large eating cell." Macrophages tend to follow neutrophils to sites of injury/inflammation. These cells, unlike neutrophils, are capable of repeated phagocytosis and have the ability to secrete copious amounts of inflammatory proteins, including cytokines. Selected macrophages also have the ability to process and present antigens. Macrophages are key innate immune cells and are one of the

important targets of estrogen within the immune system (Ansar Ahmed et al. 1999). Estrogen increases murine and human macrophage phagocytic activity (Baranao et al. 1992). Further, in mice, the percentage of macrophages in the endometrial stromal and myometrial connective tissues of the cycling uterus changes relative to the stages of the estrus cycle (De and Wood 1990).

Generally, androgens inhibit the function of macrophages in vivo and in vitro (Miller and Hunt 1996). Androgen receptors (AR) have been identified in primary cultured macrophages (Cutolo et al. 1996). Stimulation of murine macrophages with testosterone in vitro reduces the synthesis of proinflammatory products, including TNF-α and nitric oxide synthase (D'Agostino et al. 1999). Testosterone also reduces toll-like receptor (TLR) 4 expression on macrophages (Rettew et al. 2008). Testosterone attenuates *Leishmania donovani*-mediated p38MAPK activation of macrophages, which is considered to be the cause of testosterone-enhanced *L. donovani* survival in macrophages (Liu et al. 2006). Furthermore, androgens such as testosterone, DHT, mesterolone, and danazol modulate the clearance of IgG-sensitized erythocytes by decreasing macrophage FcγR expression (Gomez et al. 2000).

2.2.3 Dendritic Cells

DCs are highly potent APCs, which activate naive T lymphocytes and assist in regulation of Th1 and Th2 development. As also discussed in Chap. 3 of this book, 17β-estradiol promotes differentiation of functional DCs from precursor cells. In vitro estrogen exposure of splenic DCs from rats with experimental allergic encephalomyelitis (EAE) (Zhang et al. 2004), neural DCs from mice with EAE (Liu et al. 2002) or murine bone marrow-derived DCs (Siracusa et al. 2008) increases the expression of markers of DC activation, including major histocompatibility complex II (MHCII), CD80 (B7.1), CD86 (B7.2), and CD40. Estrogen receptor (ER) antagonists, ICI 182 780 and tamoxifen, inhibit DC differentiation (Paharkova-Vatchkova et al. 2004), which is restored by the addition of physiological concentrations of estrogen (Paharkova-Vatchkova et al. 2004). The activity of IFN-producing killer DCs (IKDCs) is increased in spleens from estrogen-treated as compared with ovariectomized C57BL/6 female mice (Siracusa et al. 2008). Estrogen-treated DCs induce IKDCs and increase nitric oxide (Zhang et al. 2004). Treatment of murine splenic DCs with estrogen increases intracellular IL-6 and IL-10 expression, but not IL-12 or TNF-α (Yang et al. 2006). Human monocyte-derived immature DCs have increased IL-6, CXCL8, and CCL2 secretion after short-term in vitro estrogen treatment (Bengtsson et al. 2004). In contrast to estrogen, testosterone decreases the production of inflammatory cytokines (including IL-1β, IL-6, and TNFα) from DCs (Corrales et al. 2006).

During pregnancy, when high levels of estrogen are evident, maturation of monocytes to mature DCs as determined by expression of CD80, CD86,