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Does immune response cause oxidative stress in birds? A meta-analysis

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ABSTRACT

In recent years ecological research has focused on the relevance of antioxidants and oxidative stress in the evolution of life-history strategies and physiological trade-off in birds. Some studies sought to evaluate whether a consequence of immune response is oxidative stress. In a meta-analysis of 16 studies of ten species of birds including 49 estimates of effect size from experimental studies, we show that induction of an immune response in a diverse group of bird species may determine oxidative stress (variance explained: 4.1%), but, most notably, may determine changes in oxidative stress markers (variance explained: 15.0%). These conclusions were robust to control for sampling effort and publication bias. Finally, this finding suggests that (1) oxidative stress may be a physiological cost associated with the immune response and (2) an important role of antioxidants in birds is to control the potentially negative effects of such oxidative stress to prevent immuno-pathological damage to host tissues.

1. Introduction

Investment in immune response may be physiologically demanding (Lochmiller and Deerenberg, 2000). Birds are in fact faced with the dilemma of investing sufficiently to mount an effective immune response, but not too much to avoid depletion of energy reserves and pathological side-effects caused by an elevated immune response. This problem of optimizing immune responses may be relevant, for example, for the relationship between life history decisions and parasitism given the trade-off between investment in life history traits (e.g., clutch size, rate of aging) and immunity (Sheldon and Verhulst, 1996; Norris and Evans, 2000). Another important factor that might modulate investment in immune response is the oxidative stress that is potentially associated with the immune response itself (Fig. 1). Oxidative stress arises from the imbalance between pro-oxidants and antioxidants in favor of the former, leading to the generation of oxidative damage (Sies, 1991; Halliwell and Gutteridge, 2007). Oxidative stress is an important factor underlying reproductive performance, cellular senescence and aging, and so is an important fitness-related trait (Beckman and Ames, 1998; Hulbert et al., 2007). Recent studies show that levels of pro-oxidants and antioxidants may also have relevant ecological and evolutionary roles and may help understand functional interactions among life history traits (von Schantz et al., 1999; Costantini, 2008; Monaghan et al., 2009).

Part of the immune response relies on immune cells that kill pathogens by releasing pro-oxidant compounds. Professional phagocytes (macrophages, eosinophils, heterophils), as well as B and T lymphocytes contain a multi-component enzyme complex, the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Hampton et al., 1998; Babior, 1999). This enzyme complex is responsible for the production of reactive oxygen species after immune stimulation.

At the onset of an immune response, phagocytes increase their oxygen uptake as much as 10–20 times the resting values (respiratory burst). The NADPH oxidase is oxidized into $NADP^+$, releasing two electrons, which are used to reduce oxygen to superoxide free radical:

$$NADPH + 2O_2 \rightarrow NADP^+ + H^+ + 2O^{\bullet -}.$$

The $O^{\bullet -}$ generated by this enzyme serves as the starting material for the production of a suite of reactive species (Fig. 1). Direct evidence indicates that immune cells also release other powerful pro-oxidants, such as hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), peroxynitrite ($ONOO^-$) and, possibly, hydroxyl ($OH^{\bullet}$) and ozone (O_3).

Independent evidence shows that pathogens with impaired antioxidant defenses are more sensitive to phagocytic killing, indicating a role for reactive oxygen species as microbicidal agents (Halliwell and Gutteridge, 2007). Further studies show that individuals deficient in oxidase are susceptible to severe bacterial and fungal infections (Nathan and Shiloh, 2000).

Reactive species are important in killing pathogens, but can as a negative side effect also damage host tissues (immuno-pathology). This is particularly evident during chronic inflammation, which may cause extensive tissue oxidative damage with a consequent increase in oxidative stress (Sorci and Faivre, 2009).

Recent studies on captive and wild birds (Table 1) have investigated the effects of immune response on oxidative stress markers, yielding contrasting results. Some studies showed an

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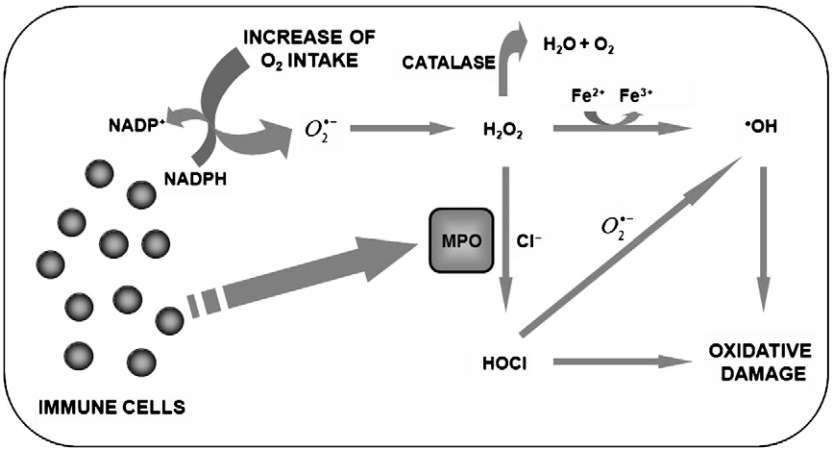

Fig. 1. The physiological pathways resulting in oxidative stress as a consequence of the cascades produced by an immune response. HOCl = hypochlorous acid; MPO = myeloperoxidase; $NADP^+$/NADPH = nicotinamide adenine dinucleotide phosphate.

increase in oxidative damage and a decrease in antioxidants following an immune challenge, while others did not. Here, we review this recent evidence, perform a meta-analysis to test the hypothesis that "immune response increases oxidative stress", and try to reconcile the contrasting results of these studies.

We will focus on birds because, compared to other taxa, the link between avian immune response and changes in oxidative stress markers has been widely studied; birds have evolved sophisticated mechanisms to mitigate detrimental effects of oxidative stress (Costantini, 2008); and birds have been excellent subjects in many areas of biological research (Konishi et al., 1989).

2. Material and methods

A systematic search of research investigating the effects of immune response on oxidative stress markers was conducted by accessing several sources (e.g., PubMed) and by checking the literature quoted in each article addressing this issue. In this paper, we review 16 studies for a total number of 49 effect size estimates of immune response on oxidative stress markers (Appendix). All studies on nitrosative stress were not considered because of the differences in biochemical roles between reactive oxygen and nitrogen species. For each study, we calculated effect size from test statistics reported in the original papers by using the equations given by Rosenthal (1991, 1994: Table 16.1).

For analyses of variance we used the *F*-value for the contrast of interest to estimate effect size, as suggested by R. Rosenthal. In a hypothetical example for four groups with a predicted linear trend in four mean values, we have weights of −3, −1, +1, and +3 associated with four means of 2, 5, 7, and 8. The correlation coefficient (r_{alerting}) between contrast weights and means is 0.976 and r^2_{alerting} is 0.952. If we assume an omnibus *F* (with 3 *d.f.* in the numerator) of 2.0, then we get *F* for contrast (an *F* with 1 *d.f.* in the numerator) from:

$$F_{\text{contrast}} = F_{\text{omnibus}} \times d.f._{\text{numerator}} \times r^2_{\text{alerting}}.$$

In the present example we get $2.0 \times 3 \times 0.952 = 5.71$.

From F_{contrast} we get r_{contrast} from

$$r_{\text{contrast}} = \sqrt{(F_{\text{contrast}})} / (F_{\text{contrast}} + d.f._{\text{error}}) = \sqrt{5.71} / (5.71 + 36) = 0.37,$$

assuming that $n = 10$ for each of the four groups.

In a first analysis, the sign of each effect size was considered to be positive when oxidative damage was increased and antioxidants were decreased after immune stimulation, but was negative when oxidative damage was decreased and antioxidants increased after immune stimulation. This allowed us to evaluate effects of immune response on oxidative stress. In a second analysis, all the effect sizes were assigned the same sign. This allowed us to evaluate to what extent the immune response determines changes in oxidative status markers, regardless of whether this causes or does not cause oxidative stress.

The measure of effect size used in the meta-analyses was Pearson's correlation coefficient. Pearson's correlation coefficients were subsequently transformed by means of Fisher's transformation to *z*-values on which subsequent analyses were performed. This measure of effect size was adjusted for sample size using $N - 3$ as an adjustment factor (Rosenthal, 1991, pp. 27–28), based on the assumption that a larger sample size should provide a more reliable estimate of the unknown true relationship. We calculated an overall effect using mean effect size adjusted for sample size after *z*-transformation. The mean weighted z_r values were tested against the null hypothesis of no effect by examining the significance of their associated *r*'s. This was done by back-transforming the mean weighted effect size to a correlation coefficient and testing the significance of this coefficient given the total sample size (Rosenthal, 1991). An estimate of heterogeneity in effect sizes among samples was subsequently calculated using the formula provided by Rosenthal (1991, pp. 73–74), which has a χ^2-distribution with $K - 1$ degrees of freedom, where *K* is the number of analysis units.

There are both direct and indirect ways of estimating publication bias (Møller and Jennions, 2004). Given that direct estimates would require knowledge about studies initiated, but never published, we were unable to pursue this approach. Instead, we estimated the fail-safe number as the number of unpublished null studies needed to eliminate the observed global effect size. We obtained that estimate by relying on the equations reported by Rosenthal (1991, p. 104).

3. Results

In a first meta-analysis based on 49 individual effect sizes we tested for the magnitude of the overall effect adjusted for sample size

Table 1
Summary statistics for meta-analyses of effects of immune response on oxidative stress and effects of immune response on oxidative status markers based of 49 estimates of effect sizes from 16 studies of birds.

	Effects of immune response on oxidative stress	Effects of immune response on oxidative status markers
z	0.2014***	0.3876***
Pearson's *r*	0.1987	0.3693
Heterogeneity in effect χ^2	318.03	118.25
d.f.	48	48
p	<0.001	<0.001
Fail-safe number	95	95