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Abstract

Cutaneous basophil hypersensitivity response to phytohemagglutinin (PHA) is frequently used as a measure of cell-mediated immune response in chickens. Several anatomical sites have been used to measure this response. No correlation between response level at different sites, however, has been reported. This study compares responses to PHA by using two different injection sites (wing web and interdigital skin of the foot) in White Leghorn chickens. Response at each individual site was comparable whether the bird received a PHA injection at that site alone or at both sites. Correlations between responses at the two sites were not different from zero [-0.1733 ($P = .44$)]. These data indicate that caution must be exercised in comparing cell-mediated immune responses when different PHA injection sites have been used to assess response.

Keywords

chicken, basophil, phytohemagglutinin, injection site, immune response

Disciplines

Agriculture | Animal Sciences | Poultry or Avian Science

Comments

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Effect of Injection Site on Cutaneous Basophil Hypersensitivity Response to Phytohemagglutinin¹

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ABSTRACT Cutaneous basophil hypersensitivity response to phytohemagglutinin (PHA) is frequently used as a measure of cell-mediated immune response in chickens. Several anatomical sites have been used to measure this response. No correlation between response level at different sites, however, has been reported. This study compares responses to PHA by using two different injection sites (wing web and interdigital skin of the foot) in White Leghorn chickens. Response at each individual site was comparable whether the bird received a PHA injection at that site alone or at both sites. Correlations between responses at the two sites were not different from zero [-.1733 ($P = .44$)]. These data indicate that caution must be exercised in comparing cell-mediated immune responses when different PHA injection sites have been used to assess response.

(*Key words*: chicken, basophil, phytohemagglutinin, injection site, immune response)

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INTRODUCTION

The localized *in vivo* inflammatory response of chickens to phytohemagglutinin (PHA) has long been used as a measurement of cell-mediated immunity (Stadecker *et al.*, 1977; Goto *et al.*, 1978). Several groups have examined response to PHA and have used several different sites of injection. Using the wattle as the site of injection, both Stadecker *et al.* (1977) and McCorkle *et al.* (1980) characterized this response as an infiltration of basophils. Stadecker *et al.* (1977) also used the skin near the ear as a site of injection. No response difference was reported. By using the wing web as the site of injection, Van der Zijpp (1983), Lamont and Smyth (1984), and Cheng and Lamont (1988) have carried out studies of cell-mediated im-

mune response. The wing web was preferred over the wattle in testing immature chickens because of the greater accessibility of the test site.

Corrier and DeLoach (1990) characterized use of the interdigital skin of the foot as an injection site in male broiler chicks of 3 to 14 d of age. Chicks as young as 3 d of age exhibited a significant response to PHA when injected at this site. Liljequist *et al.* (1992) tested the PHA response of the interdigital skin of the foot in female broilers. They reported inconsistent results from chicks at 5 d or at greater than 19 d of age, but did not examine birds older than 40 d. The interdigital skin response has neither been reported in White Leghorn chickens nor been examined in chickens older than 40 d.

No studies report on the correlations of responses at different sites of injection, perhaps because all sites have been assumed to be equivalent for measures of PHA response. The purpose of the experiment reported here was to compare and to determine the correlation between the cutaneous basophil responses to PHA in two different sites, the wing web and the

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TABLE 1. Wing web and foot web response to phytohemagglutinin (PHA)

Injection site	n ¹	Wing index ²	SEM	Foot index ³	SEM
Wing web	5	1.360	.194
Foot and wing	22	1.320	.093	.980	.185
Foot web	5957	.088
Probability ⁴		.86		.91	

¹n = number of birds per group.

²Wing index = thickness increase of PHA-injected wing - thickness increase of saline-injected wing.

³Foot index = thickness increase of PHA-injected foot - thickness increase of saline-injected foot.

⁴P value of differences of means within column.

interdigital skin of the foot, in White Leghorn chickens.

MATERIALS AND METHODS

Measurement of Cell-Mediated Immune Response

Wing web response to PHA⁴ was measured according to the method described by Cheng and Lamont (1988). One-tenth milliliter PHA (10 mg/mL) was injected intradermally into the right wing web, and .1 mL of physiological saline (PBS) was injected into the left wing web. A micrometer was used to measure the thickness of each wing web just before injection and again 24 h later. Response was recorded (in millimeters) as

$$\text{wing index} = (\text{post-PHA} - \text{pre-PHA}) - (\text{post-PBS} - \text{pre-PBS})$$

(Van der Zijpp, 1983; Lamont and Smyth, 1984).

The elicitation of a PHA interdigital foot response was adapted from the method of Corrier and DeLoach (1990): one-tenth milliliter of PHA was injected intradermally into the skin between the third and fourth digits of the right foot, and .1 mL of PBS was injected into the left foot. Response, termed the foot index, was measured in millimeters by using the same formula as that for the wing index.

Chickens

All birds were male White Leghorn chickens approximately 14 wk old. The line

was synthesized from four commercial lines in the 1960s and has been used in several immunogenetics studies (Nordskog *et al.*, 1973; Cheng and Lamont, 1988). Birds were randomly divided, in unequal numbers, into three treatment groups. Five birds received injections in the wing web only, and five birds received injections in the foot web only. To evaluate possible effects of injection at multiple sites, 22 birds received injections both in the foot web and in the wing web. Each individual bird was considered an experimental unit.

Statistical Analyses

The General Linear Models procedure of SAS[®] was used to determine least squares means and to compare the responses of birds injected at both sites, only in the wing web, or only in the skin of the foot. The product-moment correlation was determined by using the correlation procedure of SAS[®] software (SAS Institute, 1985).

RESULTS

Means of the three treatment groups (testing in the skin of the foot only, wing web only, and both sites) are presented in Table 1. In comparisons of response at each individual site, the response of birds injected at both sites did not differ from that of birds injected at single sites. The product-moment correlation between wing web and foot web responses in birds of the group receiving both injections was $-.1733$ ($P = .44$).

DISCUSSION

Responses to PHA were measured in chickens injected in one or both of two

⁴Difco Laboratories, Detroit, MI 48232-7058.

different sites (wing web or interdigital skin of the foot). There was no difference between chickens injected at a single site and those injected at multiple sites. The wing web response to PHA has been previously characterized in the same line of chickens by Cheng and Lamont (1988), who reported wing index means ranging from 1.20 to 1.59 mm. Means for wing index in the current experiments (1.36, 1.32) were within this same range. Response in the interdigital skin of the foot (foot index) has not been characterized previously in layer birds. In the current study, foot index means were only slightly less consistent between groups than were wing index means and did not differ (Table 1). The means for foot index reported here are slightly greater than those reported by Corrier and DeLoach (1990), perhaps because of differences in age and genetic makeup of the experimental animals. Chickens in the current study were White Leghorns of approximately 14 wk of age, but Corrier and DeLoach used broiler chicks 2 wk of age and younger.

An important observation from this experiment was the lack of correlation between wing index and foot index for individual chickens. Because responses at each site are consistent regardless of whether a bird received single or multiple injections, the correlation of responses at different sites was estimated on the individuals receiving injections at both sites. The estimate of correlation was small and slightly negative, but not significant (product-moment correlation $P = .44$). Several factors may influence the differences in measured response between wing and foot. Measurement errors, especially due to feathers and feather follicles on the wing web, may have occurred. Every effort was made, however, to avoid including any feathers in the measurement. Similarity of the variance of wing index and foot index suggests that inclusion of extraneous tissue in the wing measurement was not a source of variation. Because the correlation between wing index and foot index is estimated within individuals, factors such as differences in genetics and weight are eliminated as

sources of variation. Differences in skin thickness and connective tissue at the two sites, as well as distance to reservoirs of recruitable leukocytes and granulocytes, might affect the rate of infiltration of cells as well as the area of tissue into which these cells migrate.

Although both wing web and foot injection sites have been chosen by researchers as a measure of cell-mediated immune response, this study indicates that caution must be exercised in interpreting and comparing responses to PHA injection at different sites. This study demonstrated a lack of correlation in the measured response at the two sites of wing web and foot and suggests, therefore, that the *in vivo* PHA response may not be comparable for all injection sites.

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