Lymphocyte Populations and Antibody Production in Vaccinated and Non-vaccinated Pigs Challenged with *Mycoplasma hyopneumoniae*

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ASL-R1409

Summary and Implications

Mycoplasma hyopneumoniae (*M. hyo*), the cause of enzootic pneumonia in swine, is a worldwide problem. Despite the developmental vaccines that have been commercially available for several years, *M. hyo*-induced pneumonia is still a major concern to swine producers. The pathologic lesions observed with enzootic pneumonia consist primarily of a lymphoid cell infiltration of the lungs. Four groups of pigs consisting of challenge control, vaccinated and challenged, vaccinated only, and non-vaccinated and non-challenged were used in this study. Lymphocytes from the peripheral blood, lung, and bronchial lymph nodes were analyzed by flow cytometry (FACs) to determine the populations of cells activated by *M. hyo*. No significant difference in percentages of B or T lymphocytes was found between the vaccinated and non-vaccinated groups. An ELISPOT assay was used to evaluate the isotype of antibodies secreted by B lymphocytes from the same tissues. Total secreted immunoglobulin and mycoplasmal membrane specific immunoglobulin secretion were measured. A significant increase in secretion of total IgG by lymphocytes in the lungs of pigs only challenged with *M. hyo*, and vaccinated/challenged was observed. No mycoplasma-specific stimulation was observed. The mycoplasmal membrane preparation induced a non-specific stimulation by IgM secreting cells in all groups. Again, no mycoplasma-specific response was observed. These results suggest that although IgG-secreting lymphocytes are stimulated to secrete antibodies in infected pigs, the lymphocyte response observed in enzootic pneumonia may not be to the *M. hyo* membrane antigen. At this time, it is unknown whether the immune response to *M. hyo* is predominately an antibody- or cell-mediated immune response, and how these immune responses contribute to protection from enzootic pneumonia.

Introduction

Enzootic pneumonia in swine caused by *M. hyo* affects the majority of swine herds in the United States. *M. hyo* infections result in a chronic pneumonia that often leads to secondary bacterial pneumonia and decreased growth rate. With the appearance of new pathogens such as porcine reproductive and respiratory syndrome (PRRS) virus, the severity of enzootic pneumonia has apparently increased, thus becoming an even greater problem for swine producers. Immunization induces serum antibodies, but provides only partial protection against the development of enzootic pneumonia and does not prevent colonization of *M. hyo* in the lungs. This indicates that other immune mechanisms, such as cell mediated immunity or secretory immunity may need to be stimulated to achieve optimal protection against *M. hyo*. Because the influx of lymphocytes into the lungs of mycoplasma infected pigs is the primary pathologic lesion observed, identifying the populations of these lymphocytes is important. In addition, it may be possible to improve the protection against enzootic pneumonia by identifying which populations of lymphocytes need to be stimulated in vaccinated pigs compared to pigs infected with *M. hyo*.

The objectives of this study were to compare the populations of lymphocytes infiltrating the lungs of pigs infected with *M. hyo* with pigs vaccinated against *M. hyo* with a commercial bacterin and determine if the lymphocytes present in the lungs of pigs vaccinated and challenged with *M. hyo* differ from the cells which play a role in protection. In addition, the isotype of immunoglobulins secreted in response to mycoplasmal membrane antigen was measured by ELISPOT assay.

Materials and Methods

Four groups of SPF mycoplasma-free pigs were used in the study. The pigs were randomly assigned to the groups with stratification by weight and sex. Each group was housed individually in isolation rooms. Three-week-old pigs in groups B and C were vaccinated with a commercial *M. hyo* bacterin twice, two weeks apart, according to the manufacturer's directions. Three weeks after the second vaccination, the pigs in Groups A and B were challenged with 10 ml. of a 1-100 dilution of 10% crude lung homogenate containing *M. hyo*.

The pigs were necropsied on day 28 post challenge, and samples of peripheral blood, lung, and bronchial lymph node was collected from each pig. Lymphocytes were isolated from each of the samples by differential centrifugation Histopaque, followed by washing and lysis of contaminating erythrocytes. Lymphocytes then were divided into two groups of cells: 1) assayed for their expression of T and B cell surface glycoproteins using FACs analysis, or 2) resuspended in media for the in vitro ELISPOT assay to assess the number and isotype of antibody secreting cells. Lesions on lung surfaces were sketched and percentage of pneumonic lung was calculated using a Zeiss image analysis system.

Results and Discussion

Pigs in group B, which received the vaccine were not completely protected from *M. hyo* challenge. The percentage of lung tissue exhibiting lesions for pigs in group B ranged from zero to 12.6% (mean = 3.4%) as measured by image analysis. All non-vaccinated and challenged pigs receiving *M. hyo* inoculum had pneumonic lung lesions (mean = 13.9%). None of the non-challenged pigs had evidence of pneumonia.

No significant difference was found between the populations of lymphocytes obtained from pigs in any of the groups as determined by FACs analysis. The lack of response by a specific population of lymphocytes suggests that both B and T lymphocytes are stimulated by mycoplasmal antigens.

Figure 1 depicts the total number of antibody secreting cells in the peripheral blood, lung, and bronchial lymph

node identified by isotype. The lymphocytes in the lung and bronchial lymph nodes from pigs in groups A and B had significantly greater numbers of IgG secreting lymphocytes. There was also a slight increase in the number of IgM secreting cells in the bronchial lymph nodes of groups A and B. These values suggest that *M. hyo* stimulates lymphocytes to secrete predominantly IgG.

Figure 2 describes the number of antibody secreting cells responding specifically to our mycoplasma membrane preparation. There appears to be a non-specific stimulation of IgM secreting cells by the mycoplasmal membrane preparation for all groups. Previous studies have reported a non-specific mitogenicity by mycoplasmal proteins. No
significant specific response to our mycoplasmal membrane preparation was observed. There may have been a slight increase in IgA secretion by lymphocytes in the peripheral blood and the lung, however, no significant difference was observed. No stimulation to the mycoplasmal membrane antigen was observed in the groups immunized and/or challenged by *M. hyo*. The lack of antigen-specific stimulation may be due to lack of recognition of our antigen, or an inadequate amount of protein used in the ELISPOT assay. The protein concentration utilized in the assay has been shown to induce lymphocyte stimulation in previous studies. This suggests that the mycoplasma membrane may not be very immunogenic on its own. The response to other mycoplasma proteins will need to be investigated to elucidate the immune responses to *M. hyo*.

The results of this study indicate that the lymphocytes responding to *M. hyo* appear to be both B and T cells. This suggests that both the cellular and humoral immune systems proliferate in response to *M. hyo*. There were significantly more lymphocytes secreting IgG in the lungs of pigs challenged with *M. hyo*, suggesting that B cells are responding to the mycoplasmal antigens. However, the lack of a membrane-specific response suggests a possible barrier to developing effective mycoplasma vaccines. The induction of a non-specific response by IgM secreting cells suggests that the mycoplasmal membrane preparation may have some superantigen effect which also may interfere with the immune response to *M. hyo*. The development of this ELISPOT assay will allow us to begin investigating the response by specific populations of lymphocytes to various mycoplasmal proteins. The knowledge obtained in these studies should aid in the development of more effective vaccines against *M. hyo*.

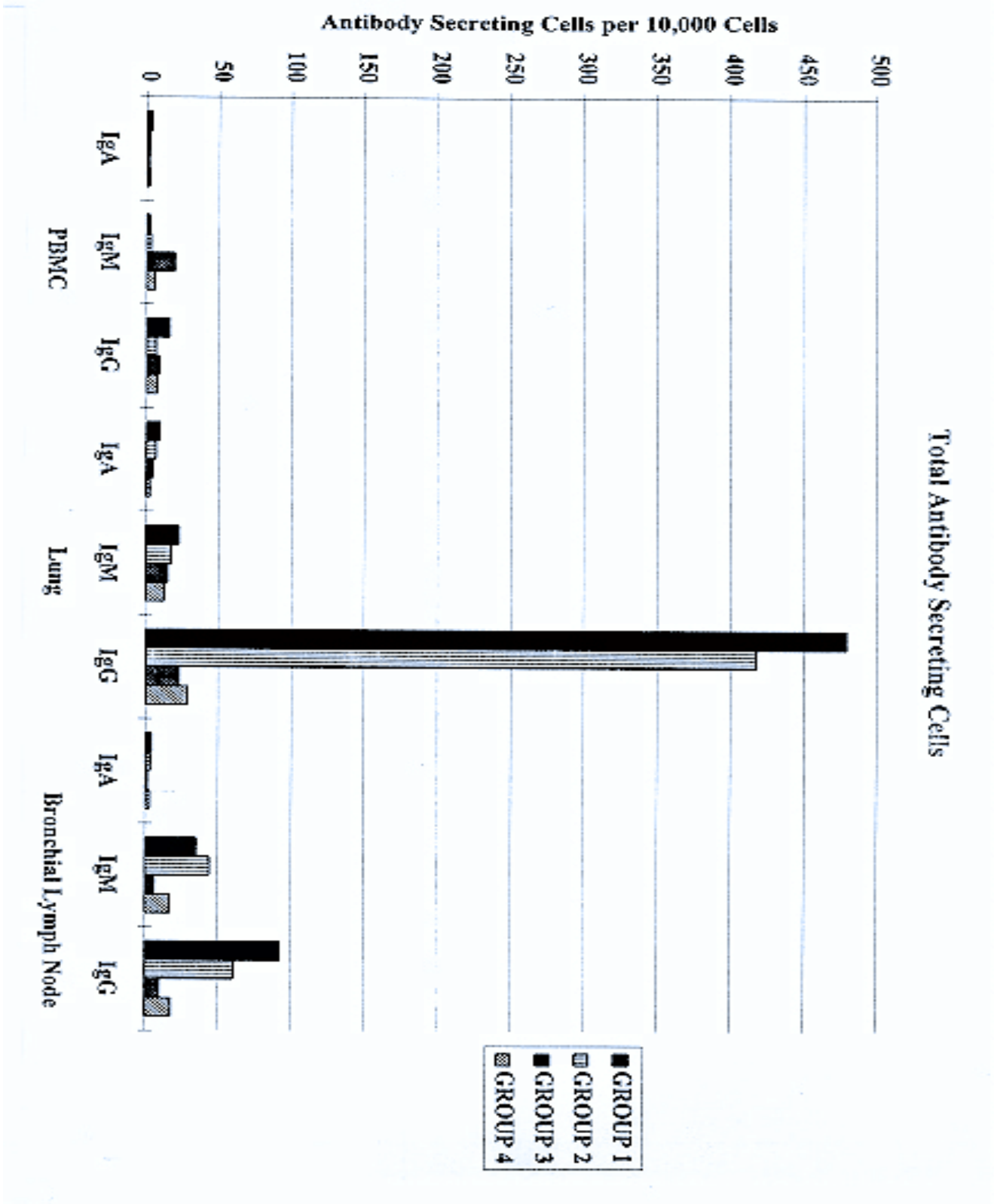

Figure 1. Number of total antibody secreting cells in the peripheral blood (PBMC), lung, and bronchial lymph node of *M. hyo* challenged (group A), vaccinated and challenged (group B), vaccinated (group C), and non-vaccinated and non-challenged (group D) pigs measured by ELISPOT assay.

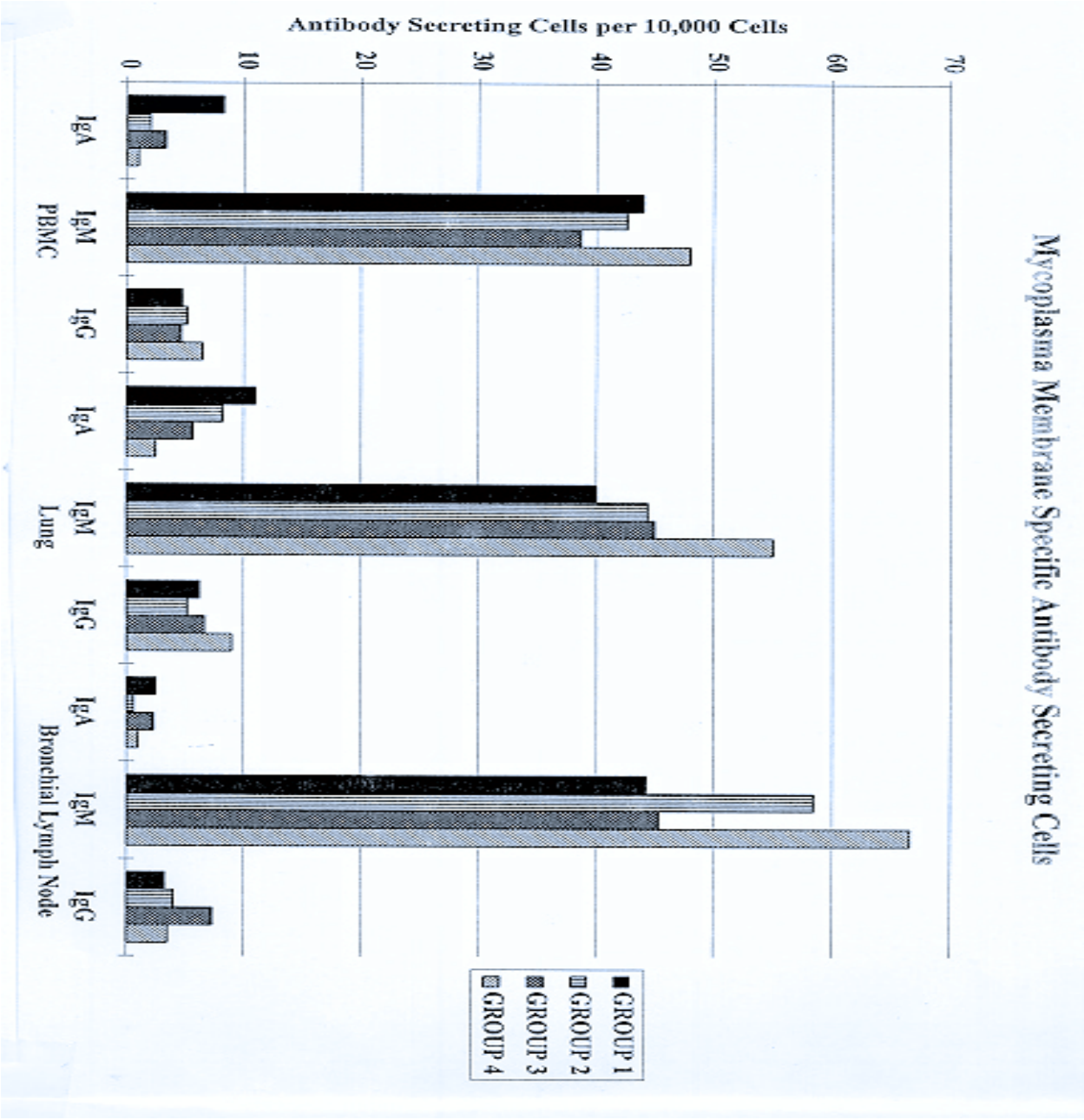

Figure 2. Number of *M. hyo* membrane specific antibody secreting cells in the peripheral blood (PBMC), lung, and bronchial lymph node of *M. hyo* challenged (group A), vaccinated and challenged (group B), vaccinated (group C), and non-vaccinated and non-challenged (group D) pigs measured by ELISPOT assay. .