Natural Antibody to Sheep Erythrocytes in Bursectomized Chickens[1] (36762)

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(Introduced by A. A. Hirata)

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Antibodies to numerous antigens in the sera of supposedly immunologically naive animals have been described. The origin and role of these "natural" antibodies are unknown, but it may be assumed that they are synthesized in a manner similar to other antibody molecules (1). This conclusion is based on the observation that natural antibody titers rise rapidly after exposure of neonatal chicks to bacteria (1) and also from the fact that neonatally thymectomized mice (2) and germ-free piglets (3) have a paucity of such natural antibodies. Natural hemagglutinin production is reportedly decreased in hormonally bursectomized chickens (4–6) and in surgically bursectomized and irradiated chickens (7). Our earlier studies, however, using newly hatched and embryonic surgically bursectomized chickens, contradict this conclusion (8). The present study was undertaken, therefore, to further evaluate antibody production, prior to antigenic challenge, by bursectomized chickens at both the cellular and serum level.

Methods. White leghorn chickens were surgically bursectomized either 2 days before hatching (19-Bx) or on the day of hatching (21-Bx) as previously reported (9). All groups of animals, including unoperated controls were housed together and chickens of each group were bled at 4 to 6 wk of age and sacrificed. The spleens were teased into single cell suspensions and washed 3 times in minimum essential medium (Grand Island Biological). These were then used in the determination of antibody production by either the immunocytoadherence or hemolytic plaque technique using sheep erythrocytes as the test antigen. The immunocytoadherence technique was performed as previously reported (8). The plaque method (10) was run essentially as modified for chicken cells by Van Alten and Meuwissen (11), with serum from a single chicken used as a source of complement. Serum hemagglutination and hemolysin levels were determined by a microtiter technique (8). Statistical evaluation of data included determination of means, standard errors of the mean, and comparison of experimental and control groups using the Student's t test and Mann-Whitney U test.

Results. Several parameters were used for evaluating immunological reactions to sheep red blood cells (S-RBC) by bursectomized chickens; the results are summarized in Table I. From these data it is obvious that positive immunological reactions occurred in both groups of bursectomized chickens as well as in the control group. It is readily evident that most bursectomized chickens, in comparison to normals, have a comparable number of splenic lymphocytes capable of binding sheep erythrocytes demonstrable by rosette formation. Similarly, the mean hemagglutinin titers of the serum of normal and 19-Bx or 21-Bx chickens are not significantly different. Further, the number of cells capable of actively secreting antibody, revealed by formation of hemolytic plaques, did not

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TABLE I. Detection of "Natural" Antibody to Sheep Erythrocytes in Normal Chickens and in Chickens Bursectomized 2 Days Before or at Hatching.

Antibody production	Normals	19-Bx	21-Bx
Rosettes	6800 ± 800[a] (29/29)[b]	7900 ± 1500 (6/7)	7300 ± 870 (14/14)
Hemagglutinin titer	0.18 ± 0.07[c] (6/30)	0.11 ± 0.06 (2/9)	0.156 ± 0.1(3/16)
Plaques	180 ± 70[a] (7/7)	73 ± 38 (3/6)	113 ± 67 (5/6)
Hemolysin titer	1.5 ± 0.098[c] (10/13)	1.47 ± 0.187 (5/8)	0.83 ± 0.15 (5/9)

[a] Mean number of cells responding ± standard error of mean per 10^6 spleen leukocytes. Statistical comparison using Student's t test and the Mann-Whitney U test indicated no significant difference between means of either 19-Bx or 21-Bx and controls ($p > 10$).

[b] Number responding/number tested.

[c] Mean titer ($\log_2$) ± standard error of mean. Statistical comparison using Student's t test and the Mann-Whitney U test indicated no significant difference between means of either 19-Bx or 21-Bx and controls ($p > 10$).

differ significantly for 19-Bx and 21-Bx chickens when compared with the normal group (Table I). Likewise, the serum hemolytic titer of bursectomized groups was not significantly different than controls.

These results show that surgical bursectomy even prior to hatching does not significantly alter either the number of splenic lymphocytes capable of reacting immunologically with sheep erythrocytes or the amount of serum antibody present prior to immunization with this antigen. On the other hand, it has been demonstrated that bursectomy, both before and at hatching depresses the ability of chickens to respond normally when immunized with S-RBC (8, 12–14).

Discussion. Previously we (8) reported reduction in the number of rosette forming cells and serum hemagglutination titers when chickens were bursectomized as embryos and subsequently challenged with S-RBC. It was unexpected, therefore, to observe that nonimmunized 19-Bx and 21-Bx chickens had comparable numbers of cells forming rosettes and hemolytic plaques and had similar amounts of serum hemagglutinins and hemolysins as found in normal chickens. Others have shown that chickens surgically bursectomized (7, 15, 16), those with a genetic dysgammaglobulinemia (17), and some hormonally bursectomized (18) have normal or even increased concentrations of serum IgM. These observations indicate that such chickens may have a sufficient number of cells to synthesize IgM.

Springer and others (1, 19) present evidence that "natural" antibodies result from animals encountering bacteria. The natural antibodies detected in bursectomized chickens may indeed be cross-reacting antibodies to microorganisms and most likely the primarily high efficient hemolytic immunoglobulin (IgM). Even in germ-free mice the presence of natural occurring plaque forming cells to S-RBC, in numbers comparable to those of conventionally reared mice, was attributed to stimulation by dead microorganisms and endotoxins present in the sterilized food, water and bedding (20). Thus, it is only following immunization that bursectomy results in the failure of specific antibody response (8). This suggests that there may be a marked reduction of antigen-reactive cells with the consequence that cellular proliferation, specific antibody and IgG production are markedly curtailed following antigenic stimulation at 4 or more wk of age.

Recently it was suggested that the lymphocytes of unimmunized human fetuses, forming rosettes with S-RBC, were of thymic origin (21). In the chicken, however, immunologically active lymphocytes may possibly have 3 sources, namely, thymus, bursa or bone marrow. In a number of thymectomized chickens, however, we (unpublished observations) have not detected any decrease in the

number of spleen lymphocytes forming either rosettes or hemolytic plaques. Therefore, it is highly unlikely that splenic lymphocytes of nonimmunized, bursectomized chickens, reacting with S-RBC are of thymic origin. It must, however, await further investigation to ascertain the precise cellular nature of the defective immunological response observed in the surgically bursectomized chicken.

Summary. Chickens surgically bursectomized 2 days before hatching or on the day of hatching were assayed at 4 to 6 wk of age for their ability to react with sheep erythrocytes. Bursectomized and normal, unoperated chickens were tested using immunocytoadherence technique, hemolytic plaque, serum hemagglutinin and hemolysin assays. These tests showed that both groups of bursectomized chickens possessed "naturally" occurring rosette forming and hemolytic plaque producing spleen cells in numbers comparable to those of normal chickens. The serum hemagglutinin and hemolysin titers also were of the same magnitude in bursectomized and normal chickens. From our results we suggest that bursectomy may deplete a population of antigen-reactive cells which are necessary for the propagation of a normal response to antigenic stimulation at 4 or more wk of age.

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