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Antigenic Specificities of Bursal and Thymic Lymphocytes in the Chicken

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Summary. Bursal and thymic antigenic specificities have been shown in the chicken in an immune adherence assay using cross-absorbed rabbit antisera.

INTRODUCTION

Antigens specific to a particular lymphoid population have already been described in mammals by several workers. Thus the TL antigens (Old, Boyse and Stockert, 1963) have been found only in the thymus of certain strains of mice and in murine leukaemic cells. In rats, a thymocyte-specific antigen (Potworowski and Nairn, 1967a, b) and a lymphoid-specific antigen present in all lymphocytes except those of the marrow (Potworowski and Nairn, 1968) have been described. In birds, despite the fact that the thymus and the bursa of Fabricius play a distinct role in the ontogenesis of the immune response (reviewed by Glick, 1969), as far as we know, no antigenic differences between thymic and bursal lymphocytes have been reported.

In the present work, cross-absorbed rabbit antisera were used, in an immune adherence assay, to distinguish bursal and thymic antigenic specificities.

MATERIAL AND METHODS

Viable cell suspensions or microsomal fractions of thymic and bursal lymphocytes from Leghorn chicken were used as immunogenic preparations. Anti-thymus and anti-bursa sera were produced in New Zealand rabbits according to three different immunization procedures:

(1) Two doses of 10^8 viable bursal or thymic lymphocytes from 1-day-old chicks, in Hanks's balanced salt solution, were injected intravenously at 2-week intervals.

(2) Two doses of 10^8 viable bursal or thymic lymphocytes from 1-day-old chicks, in Hanks's balanced salt solution, were injected. The first dose was mixed with Freund's complete adjuvant and injected into the four footpads, the second dose was injected intravenously 2 weeks later.

(3) Microsomal fractions of thymus or bursa from 4-week-old chicks were prepared as described by Whitbeck and Rosenberg (1964). Six doses, each containing 1 mg of protein, were injected with Freund's complete adjuvant intramuscularly at weekly intervals.

In all cases, the rabbits were bled 1 week after the last injection. The globulins were precipitated from the sera by adding an equal volume of a saturated ammonium sulphate solution. The antibody preparations were then absorbed with normal chicken serum.

TABLE 1

IMMUNE ADHERENCE TITRES OF ANTI-BURSA AND ANTI-THYMUS SERA BEFORE AND AFTER CROSS-ABSORPTION

Immunogenic preparation	Absorption of antisera with lymphocytes from	Immune adherence titres	
		Thymic cells (15×10^3/mm^3)	Bursal cells (10×10^3/mm^3)
Bursal viable lymphocytes	–	1024	512
	Thymus	0	512
	Bursa	0	0
Bursal viable lymphocytes + FCA*	–	512	512
	Thymus	0	256
Bursal microsomal fraction + FCA	–	2048	16248
	Thymus	0	1024
Thymic viable lymphocytes	–	8192	1024
	Bursa	4096	0
	Thymus	0	0
Thymic viable lymphocytes + FCA	–	2048	256
	Bursa	4096	0
Thymic microsomal fraction + FCA	–	512	512
	Bursa	128	0

* Freund's complete adjuvant.

The anti-thymus sera were rendered thymocyte-specific by repeated absorptions with packed bursal lymphocytes until their immune adherence reactions with the latter were negative. A similar absorption with thymic lymphocytes was done on anti-bursa sera.

The immune adherence assay was done according to the technique of Nelson (1953) modified by Melief, van der Hast, Engelfriet and van Loghem (1967) with bursal or thymic lymphocytes as antigens, using human erythrocytes as indicator cells and fresh guinea-pig serum as a source of complement. The tests were performed using serial twofold dilution of the antisera in Microtiter plates (Cooke Engineering, Alexandria, Virginia) by a micromethod (Cooper, 1969). Maximal sensitivity of the reaction was obtained when the concentration of thymic cells was $15 \times 10^3/mm^3$ and that of the bursal cells $10 \times 10^3/mm^3$.

RESULTS

The positive immune adherence reactions (Table 1) obtained with all antisera against thymic or bursal lymphocytes indicate that some antigenic determinants are shared by the lymphocytes from both these organs. The titres with thymic and bursal cells were either similar to each other within one dilution, or stronger in the case of the homologous reaction.

The anti-bursa sera, when repeatedly absorbed with thymic cells until they became non-reactive with the latter, could still react with bursal lymphocytes. Similarly, anti-thymus sera, absorbed with bursal cells, could still react with thymocytes. These cross-absorptions seem to remove antibodies corresponding to the antigenic components common to both bursa and thymus, without appreciably affecting the titre of the homologous antibodies, except in the case of anti-microsomal sera, where the homologous titres were significantly lowered by absorption. Homologous absorption of both anti-thymus and anti-bursa sera removed all their immune adherence activity.

The specificity of anti-thymus and anti-bursa sera was demonstrated equally well, whether the immunogenic cell preparation was from 1-day-old or from 4-week-old chicken. Similarly, the specificity of the sera obtained did not seem to be appreciably influenced by the immunization schedule used.

DISCUSSION

The results we have presented show that the lymphocytes of the thymus and of the bursa of Fabricius can be identified by their respective specific antigenicities. The fact that these specific antigenicities were demonstrated by immune adherence suggests that they are associated with the cell membrane. Indeed, Melief *et al.* (1967) have observed a close relationship between the immune adherence assay and the cytotoxicity test in the measure of antibodies against lymphocytes. Positive immune adherence by anti-microsomal sera could reflect the existence of similar antigenic determinants on the cell membrane and in the intracytoplasmic vesicles present in the microsomal fraction.

The present work shows that lymphocytes from the bursa of Fabricius and from the thymus, which are known to play a distinct role in the ontogenesis of the immune response, can be distinguished by their surface antigens.

Studies are now in progress to selectively inhibit bursal or thymic dependent immunological functions by the appropriate antiserum, and to use a serological approach in the search for a mammalian equivalent to the bursa of Fabricius.

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