Natural Lymphocytic Rosettes in Non-Operated, Thymectomized and Bursectomized Chickens

This paper compares the level of spontaneous[1] or natural rosette-forming cells (RFC) in the thymus, bursa of Fabricius, spleen and bone marrow, and the effect of neonatal bursectomy and thymectomy on the formation of natural lymphocytic and nonlymphocytic rosettes in the chicken.

Methods. Non-operated, and neonatally bursectomized and thymectomized White Rock chickens, which did not experience immunization with guinea-pig red blood cells (GPRBC), were used in the experiment at 8 weeks old. The completeness of bursectomy and thymectomy was controlled grossly and histologically. In addition, serial sections of the thyroid region of thymectomized birds were inspected for the presence of extra thymic lobes. Bursaless chickens were not treated with cyclophosphamide, since the preliminary study showed that this cytotoxic agent affects also thymus-dependent (T) lymphocytes. Cells capable of forming rosettes with GPRBC were detected in the thymus, bursa, spleen and femoral marrow. For this purpose, 5×10^6 viable cells were suspended in HANKS' solution and mixed with 5×10^7 GPRBC. The mixture was centrifuged ($100 \times g$ for 15 min) and incubated for 30 min at 4°C. The cells were then gently resuspended and rosettes were counted in a haemocytometer. Cells surrounded by 5 or more GPRBC were considered as RFC. The monolayer smears obtained by centrifugation (Shandon microcentrifuge) of RFC-containing samples directly onto slides were stained with May-Gruenwald and Giemsa, and the morphology of RFC defined[2]. A goat anti-chicken Ig serum previously absorbed with chicken and guinea-pig erythrocytes was used for the inhibition of rosette formation. 2.5×10^6 thymus, bursa, spleen or marrow cells in 0.5 ml of Hanks' solution were mixed with 0.1 ml of anti-Ig or normal absorbed goat serum, and incubated for 30 min at 20°C. The test cells were then washed twice before the addition of GPRBC. Natural haemagglutinins against GPRBC in the sera of chickens were determined by means of a microhaemagglutination reaction before and after treatment of sera with 2-mercaptoethanol. Student's *t*-test was employed for statistical analysis.

Results. The mean titer ($\log_2 \pm$ SE) of natural anti-GPRBC agglutinin of IgM type in 12 non-operated chickens was 3.08 ± 0.44. The level of natural antibody drastically decreased in 10 neonatally bursectomized (0.50 ± 0.97), but not in 18 thymectomized birds (2.83 ± 0.97). Cytomorphological analysis of RFC revealed that the great majority of RFC was of the lymphocytic class, and that the number of those RFC was significantly higher in the spleen and bone marrow than in the bursa and thymus (Table). GPRBC clustered around granulocytes were regularly seen in all preparations of the spleen and bone marrow. The latter contained also rosettes composed of chicken erythroblasts and GPRBC.

There was a marked decrease in the number of lymphocytic rosettes in the spleen and marrow of both bursectomized and thymectomized chickens, and in the thymus and bursa of bursaless and thymusless birds respectively. On the other hand, neither bursectomy nor thymectomy influenced the population of nonlymphocytic rosettes.

Complete inhibition of rosetting reaction was consistently obtained by incubation of bursal and thymic cells with anti-Ig serum. This inhibition failed, however, when anti-Ig serum absorbed with chicken Ig was used

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Effect of bursectomy and thymectomy on naturally occurring RFC, and inhibition of rosette formation with goat anti-chicken Ig serum

Source of cells	Treatment[a]	No. of RFC per 10^6 cells (Mean ± SE) Total number	Type of RFC Lymphocytic	Granulocytic	Erythroblastic	Percentage of inhibition with anti-Ig (range and mean)
Spleen	None	2650 ± 351	2547 ± 246 (96.1)	103 ± 18 (3.9)	0 —	78–98 88.1
	Bx	846 ± 183[b]	778 ± 165[b] (92.0)	68 ± 19 (8.0)	0 —	20–97 60.2
	Tx	1011 ± 169[b]	939 ± 168[b] (92.9)	72 ± 23 (7.1)	0 —	50–92 85.7
Bone marrow	None	3350 ± 318	1963 ± 165 (58.6)	945 ± 97 (28.2)	442 ± 39 (13.2)	50–72 64.1
	Bx	2235 ± 325	1031 ± 216[b] (46.2)	831 ± 184 (37.1)	373 ± 106 (16.7)	34–88 65.4
	Tx	1890 ± 190[b]	967 ± 103[b] (51.1)	644 ± 158 (34.1)	279 ± 87 (14.8)	29–85 80.8
Bursa	None	320 ± 77	318 ± 87 (99.4)	2 ± 0.4 (0.6)	0 —	— 100
	Tx	111 ± 48[b]	108 ± 31[b] (97.1)	3 ± 0.9 (2.9)	0 —	— 100
Thymus	None	50 ± 10	49 ± 15 (97.9)	1 ± 0.6 (2.1)	0 —	— 100
	Bx	0[b]	0[b]	0	0	N.D.

[a] No. of chickens: 12 nonoperated (None), 10 bursectomized (Bx) and 18 thymectomized (Tx). [b] Statistically significant difference ($p < 0.05$). Mean percentage of RFC in parenthesis.

in the reaction, thus indicating that the blockade of rosette formation was due to a specific anti-Ig component of the antiserum. The inhibition was less effective in the case of spleen and bone marrow.

Discussion. In the chicken, the appearance of RFC is considered a bursa-dependent phenomenon[3]. However, the most interesting finding of this study is the striking decrease in the number of lymphocytic RFC in the bursa, spleen and bone marrow of thymectomized birds thus suggesting the existence of a thymus-dependent RFC. The T rosettes[4], and 2 populations of thymus-derived RFC, 1 of them being influenced by thymectomy[5], have been described in mice. Rosette formation by T cells was also inhibited by anti-Ig reagent[6]. The involvement of the chicken thymus in the formation of rosettes can be explained in at least 2 ways. The first possibility is than T cells interact with bursa-dependent cells (Bu cells) it rosette reactions so that T cells exert a helper function whereas Bu cells establish a more substantial contact with erythrocytes[7]. The second assumption is that T cells are themselves capable of forming rosettes, and this is probably the case with lymphocytic rosettes in bursaless birds. Both explanations, of course, concern RFC in non-immunized chickens, and one may expect a somewhat different situation in birds immunized with GPRBC.

Although it has been stated that natural RFC constitute progenitors of antibody-producing cells[8], the immune nature of those rosettes is still unclear. As has been proposed for natural haemagglutinins and haemolysins[9], the natural RFC may also arise from previous contacts of the animal with cross-reacting antigens, or their appearance is under genetic control.

Ig receptors have been demonstrated on the surface of chicken Bu cells[3,10]. However, the existence of Ig receptors on mammalian and avian T cells is the subject of divergent views, since both the presence[5,11] and the absence[12] of Ig receptors have been reported. Although our results favour the former view, it still remains to be seen whether natural lymphocytic rosettes in the chicken are an expression of Ig-like receptors on lymphocytes[13], or a special class of 'physiological' rosettes similar to those which human T cells form with sheep erythrocytes[14].

The possibility that T rosettes may be formed 'pasively' via a cytophilic antibody elaborated by Bu cells[15] seems unlikely since there was a clear difference between spleen, marrow and thymus lymphocytes of bursectomized birds with respect to their ability to produce rosettes. Finally, the formation of rosettes by granulocytes and erythroblasts remains an event which can be treated as a physico-chemical, a physiological, a developmental or an immunological phenomenon.

Résumé. On démontre la présence des lymphocytes capables de faire des rosettes spontanées ou naturelles avec les globules rouges de cobaye, dans le thymus, la bourse de Fabricius, la rate et dans la moelle osseuse des poulets non-immunisés, âgés de 8 semaines. Les effets de la boursectomie et de la thymectomie néonatale chez le poulet, suggèrent l'existence de 2 populations de lymphocytes capables de faire des rosettes, dont l'une thymo-dépendente et l'autre dépendant de la bourse.

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The Location of Soluble Antigen in the Spleen of *Xenopus laevis*

The pattern of antigen localization in the spleen has been traced in rats[1], *Bufo marinus*[2] and mice[3] using flagellar antigens or aggregated human γ-globulin (HGG). It is known from previous studies by Horton and Manning[4] using a fluorescence technique to detect antigen, that *Xenopus laevis* – an animal lacking in lymph nodes – can trap antigen in its spleen. They found that 3 weeks after injecting HGG in adjuvant, the fluorescent picture was diminished or absent in thymectomized toadlets. In this report, the location of antigen in spleens from intact and thymectomized *Xenopus* injected with HGG in saline was investigated at various time intervals after injection. The antigen was traced using a fluorescein labelled antiserum to HGG.

Materials and methods. The experimental animals (weighing approximately 8 g and maintained at 20 °C) consisted of 56 intact, 10 thymectomized and 7 sham-thymectomized toadlets. For details of the thymectomy operation at larval stage 48 of Nieuwkoop and Faber[5] see Horton and Manning[4]. A standard solution of 5 mg of HGG (Kabi, Stockholm) in 1 ml of 0.85% saline was prepared and injected in various volumes via the dorsal lymph sac so that toads received 0.1, 0.15, 0.6 or 1.5 mg HGG in 1 injection. 28 control animals received saline only. Toads were sacrificed at 1, 6, 12 and 24 h, then daily for 1 week then every 2 weeks for 10 weeks after injection. The spleen was removed, quick-frozen in liquid nitrogen and sectioned at 10 µm on a cryostat. The immunofluorescent technique for the detection of antigen[4] was applied to the spleen sections. Serum, from toads killed 8 or 10 weeks after receiving 0.6 or 1.5 mg HGG was tested for

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