

EFFECTS OF LOCAL AND SYSTEMIC ADMINISTRATION OF ANTI MACROPHAGE SERUM ON POST CAPILLARY VENULES IN THE BURSAL T CELL AREA OF SRBC-IMMUNIZED CHICKENS

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ABSTRACT

The effects of rabbit anti chicken macrophage serum (RACMS) on the morphology of the diffusely infiltrated area (DIA; the bursal T cell region) of chickens stimulated locally (per anum) with SRBC (a T cell dependent antigen) were studied morphometrically.

Local administration of RACMS caused pronounced morphological alterations in the post capillary venules (PCVs) of the DIA in SRBC-immunized chickens; significant decrease ( $p < 0.001$ ) in the diameter of the PCV ( $D_{pcv}$ ) and its lumen ( $D_{lu}$ ) due to the flattening of the endothelial cells. The decrease in the migration index was significant ( $p < 0.001$ ) as well. Local administration of RACMS decreased the percentage of MPS cells to some degree ( $p < 0.02$ ), whereas systemic administration of RACMS caused a highly significant decline ( $p < 0.001$ ) in the percentage of these cells.

This study confirms the important function of MPS cells in a T-dependent immune response, and further suggests that also circulating MPS cells like T and B lymphocytes may use the PCVs as route of migration from blood to lymphatic tissue.

INTRODUCTION

The chicken cloacal bursa (of Fabricius) is a lympho-epithelial organ responsible for the differentiation of

B lymphocytes (Toivanen and Toivanen, 1973, Toivanen et al., 1974). Using histochemical methods, however, a distinct T cell region, the diffusely infiltrated area (DIA) containing 18.8% T lymphocytes has been found in the bursa (Odend'hal and Breazile, 1979a, 1980, Odend'hal and Player, 1979). The DIA is characterized by high-endothelial post capillary venules (PCVs) that undergo structural changes in response to local (per anum) immunization with sheep red blood cells (SRBC), a T cell dependent antigen (Syrjänen and Naukkarinen, 1982). This finding is analogous to that observed in the PCVs of mammalian lymphatic tissues (Kruger, 1968, Ford, 1975, Syrjänen, 1978, Kittas and Henry, 1981). Furthermore, local treatment with anti T lymphocyte serum (RACTS) preceding SRBC stimulation causes prominent changes in the DIA, thus simulating the situation in mice (Cottier et al., 1973, Syrjänen, 1978, 1982). Following RACTS treatment, the diameter of the PCV and its lumen decreased significantly due to the flattening of the endothelial cells. As expected, the number of T lymphocytes decreased, but so did also that of MPS cells, i.e. macrophages (Naukkarinen and Syrjänen, 1984).

Macrophages are known to collaborate T cells in the antibody-mediated immune response, but studies in mammals on the immunological importance of macrophages by depleting them with anti macrophage serum treatment have given controversial results (Unanue, 1972). Although the effects of anti T lymphocyte serum on the morphology of the T cell areas are well documented (Syrjänen, 1978, 1982, Naukkarinen and Syrjänen, 1984) the corresponding effects of anti macrophage serum have not been studied.

Based on morphometrical measurements, it is shown in the present study that locally (per anum) administered rabbit anti chicken macrophage serum (RACMS) causes prominent structural changes in the DIA of SRBC-immunized chickens.

## MATERIAL AND METHODS

### Experimental animals

Male White Leghorn chickens and male New Zealand White rabbits were used when aged 4 weeks and 4.5 months, respectively. The animals were fed with commercial chicken and rabbit food and water ad libitum.

### Preparation of RACMS (rabbit anti chicken macrophage serum)

Peritoneal macrophages were obtained five days after an intraperitoneal injection of 5 ml of 3% thioglycollate medium (Thioglycolat-Bouillon, Merck, Darmstadt, Germany). The peritoneal cavity was washed a few times with phosphate

buffered saline (PBS). After centrifugation 0.5 ml of the suspension containing  $10^6$ - $10^7$  cells was mixed with an equal amount of complete Freund's adjuvant (Difco Laboratories, Detroit, Michigan, USA), and injected subcutaneously into the hind leg of the rabbit. This procedure was repeated three times at two-week intervals. The serum sampled one week after the last injection was heat-inactivated ( $+56^\circ\text{C}$ ) and adsorbed with chicken red blood cells and non-adherent spleen cells. The serum caused lysis at more than 90% of chicken peritoneal macrophages in a dilution 1:32, but less than 10% of chicken bursal or thymic lymphocytes when tested undiluted, in an *in vitro* microcytotoxicity test with guinea pig complement (Yoshida et al., 1983). When applied to chickens RACMS was used undiluted.

Serum from unimmunized rabbits (RS) was treated like RACMS and used to control the possible effects of *in vivo* injected rabbit serum on the immunological state of the chickens.

#### Application procedures

Eight series of animals (five chickens in each) were studied, four of which (1A, 1B, 2A, 2B) served as controls. All the chickens were immunized with a 10% suspension of SRBC.

The chickens in Series 1A were given 200  $\mu\text{l}$  RS per anum 6 hrs before per anum immunization with 200  $\mu\text{l}$  SRBC applied on the surface of the anal lips once a day for five consecutive days as described in detail by Sorvari et al. (1975). The chickens in Series 3A were treated as those in 1A except that RS was replaced by RACMS. The chickens in Series 2A were given RS i.v. and those in Series 4A RACMS i.v. followed by per anum immunization with SRBC. In all series B, the application procedures used in the corresponding series A were repeated once starting from the day 3 after the termination of the first set of applications.

#### Sampling and measurements

Sampling of the bursae, staining of the specimens and morphometrical measurements were carried out exactly in the same manner as described in the previous papers (Syrjänen and Naukkarinen, 1982, Naukkarinen and Syrjänen, 1984).

#### RESULTS

The effects of local and systemic treatment with RACMS combined with local SRBC stimulation on the number of MPS, B, and T cells in the DIA are summarized in Table 1. After systemic treatment with RACMS (Series 4A and 4B), the number

of MPS cells was very significantly ( $p < 0.001$ ) lowered in the per anum stimulated chickens when compared with their appropriate controls (2A and 2B, respectively). Local treatment with RACMS (Series 3A and 3B), therefore, did not significantly decrease the number of MPS cells from the control values (Series 1A and 1B, respectively).

The parameters measured and those calculated in the PCVs of the DIA are summarized in Table 2. As evident, the local RACMS treatment combined with subsequent per anum immunization with SRBC caused pronounced morphological alterations in the PCVs of the DIA: decrease in the diameter of the PCV ( $D_{pcv}$ ) and its lumen ( $D_{lu}$ ) due to the flattened endothelium. The height of the PCV endothelium ( $H_{end}$ ) was lowered from  $4.33 \pm 0.30 \mu\text{m}$  to  $3.37 \pm 0.19 \mu\text{m}$  ( $p < 0.001$ ), and from  $4.38 \pm 0.23 \mu\text{m}$  to  $3.21 \pm 0.23 \mu\text{m}$  ( $p < 0.001$ ) in Series 1A to 3A, and 1B to 3B, respectively. The migration index (MI) decreased as well; from  $2.09 \pm 0.21$  to  $1.33 \pm 0.11$  ( $p < 0.001$ ), and from  $2.56 \pm 0.21$  to  $1.94 \pm 0.14$  ( $p < 0.001$ ).

Table 1. Percentages of the cells in the bursal DIA of SRBC-immunized and RS/RACMS-treated chickens

SERIES	MPS CELLS (M $\pm$ SEM)	T CELLS (M $\pm$ SEM)	B CELLS (M $\pm$ SEM)
1A	$1.32 \pm 0.13$	$8.46 \pm 0.81$	$90.22 \pm 0.82$
1B	$1.34 \pm 0.24$	$9.44 \pm 0.49$	$89.22 \pm 0.64$
2A	$2.22 \pm 0.35$	$8.68 \pm 0.31$	$89.10 \pm 0.23$
2B	$1.92 \pm 0.27$	$8.28 \pm 1.01$	$89.80 \pm 0.99$
3A	$0.78 \pm 0.12$	$7.38 \pm 0.19$	$91.84 \pm 0.29$
3B	$0.60 \pm 0.14$	$8.50 \pm 0.88$	$90.90 \pm 0.86$
4A	$0.54 \pm 0.06$	$9.94 \pm 0.82$	$89.52 \pm 0.85$
4B	$0.36 \pm 0.08$	$7.98 \pm 0.80$	$91.66 \pm 0.88$

Table 2. Parametres assessed in the PCVs of the bursae SRBC-immunized and RS/RACMS-treated chickens

SERIES	MI (M $\pm$ SEM)	$D_{pcv}$ , $\mu\text{m}$ (M $\pm$ SEM)	$D_{lu}$ , $\mu\text{m}$ (M $\pm$ SEM)	$H_{end}$ , $\mu\text{m}$ (M $\pm$ SEM)
1A	$2.09 \pm 0.21$	$18.14 \pm 0.76$	$9.01 \pm 0.29$	$4.33 \pm 0.30$
1B	$2.56 \pm 0.21$	$21.42 \pm 1.57$	$12.44 \pm 1.04$	$4.38 \pm 0.23$
2A	$2.23 \pm 0.38$	$17.35 \pm 1.27$	$9.91 \pm 1.27$	$3.73 \pm 0.32$
2B	$2.22 \pm 0.22$	$15.75 \pm 1.40$	$9.73 \pm 1.41$	$3.15 \pm 0.17$
3A	$1.33 \pm 0.11$	$15.44 \pm 0.84$	$8.67 \pm 0.76$	$3.37 \pm 0.19$
3B	$1.94 \pm 0.14$	$18.69 \pm 1.04$	$12.14 \pm 0.63$	$3.21 \pm 0.23$
4A	$1.75 \pm 0.10$	$18.60 \pm 0.60$	$12.21 \pm 1.07$	$3.12 \pm 0.03$
4B	$1.95 \pm 0.08$	$19.30 \pm 0.66$	$12.73 \pm 0.55$	$3.28 \pm 0.07$

Explanation of the symbols: MI, migration index;  $D_{pcv}$ , diameter of a postcapillary venule (PCV);  $D_{lu}$ , diameter of a PCV lumen;  $H_{end}$ , height of the PCV endothelium

## DISCUSSION

A T-dependent antigen like SRBC is not by itself able to trigger antibody response in T helper lymphocytes, but it has to be processed and presented in an immunogenic form by MPS cells (Howie and McBride, 1982). When entering the body, antigens are usually first taken up by MPS cells. In the chicken bursa, however, the follicle-associated epithelium (FAE) does not transport SRBC into the follicular medulla where macrophages could process this antigen (Naukkarinen, 1982). Where in the bursa the antigenic information of SRBC is delivered is at present unknown. The lymphocytes in the bursal lumen (Odend'hal and Breazile, 1979b) or the FAE-like epithelium in the DIA (Syrjänen and Naukkarinen, unpublished observations) could possibly act as such sites of contact.

Antiserum against MPS cells has commonly been raised using murine peritoneal macrophages as the antigen (Unanue, 1972; Thompson et al., 1982). In chicken, peritoneal macrophages are more laborous to obtain because of the sparse amount of ascites fluid developed following thioglycollate injection, but immunization with these cells succeeds well.

In the bursal DIA, the endothelium of the post capillary venules (PCVs) hypertrophies in response to local immunization with SRBC (Syrjänen and Naukkarinen, 1982). Local treatment with anti T lymphocyte serum (RACTS) preceding local SRBC stimulation, in turn, flattens the PCV endothelium, and besides depleting T cells also decreases the number of MPS cells in the DIA (Naukkarinen and Syrjänen, 1984). This would suggest that while T cells are destroyed by RACTS there is no need for MPS cells to be recruited from blood into the DIA. Like RACTS, also the anti macrophage serum (RACMS), when administered locally, very significantly lowered the PCV endothelium. In addition, the RACMS treatment decreased the migration index (MI, Table 2) as well. This further suggests that MPS cells might utilize PCVs in their passage from blood to lymphatic tissue. Macrophages are known to secrete factors which promote vessel growth and stimulate mitosis of endothelial cells (Baldwin, 1982), but data on their circulatory properties are scanty.

In conclusion, the present observations strongly support the view that the chicken bursa exerts functions of a peripheral lymphatic organ. Further studies are needed to elucidate the dynamics of B, T, and MPS cell recirculation in the bursal DIA.

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