ACTA STEREOL 1983; 2: 438-444
STEREOLOGY AND MORPHOMETRY IN PATHOLOGY
Proc 2nd Symp Morphometry in Morphol Diagnosis
Collan Y. et al., eds.
Kuopio University Press, Kuopio 1983

# POST CAPILLARY VENULES IN THE BURSAL T CELL AREA OF SRBC-IMMUNIZED CHICKENS FOLLOWING LOCAL AND SYSTEMIC TREATMENT WITH ANTI T LYMPHOCYTE SERUM

Anita H.M. Naukkarinen and Kari J. Syrjänen

Department of Pathology, University of Kuopio, P.O.Box 6,
SF 70211 Kuopio, Finland

## ABSTRACT

The effects of rabbit anti chicken T lymphocyte serum (RACTS) on the morphology of the diffusely infiltrated area (DIA; the bursal T cell region) of chickens stimulated either locally (per anum) or systemically (i.p.) with SRBC (a T cell dependent antigen) were studied morphometrically.

Local administration of RACTS caused prominent changes in the DIA of chickens immunized locally with SRBC. The percentage of T cells decreased very significantly ($p<0.001$) as did also that of MPS cells. The following morphological alterations in the PCVs of the DIA were encountered: decrease in the diameter of the PCV ($D_{PCV}$) and its lumen ($D_{lu}$) due to the flattening of the endothelial cells. In chickens, systemically immunized with SRBC, on the other hand, the percentage of T cells decreased significantly following the systemic RACTS treatment, whereas no changes in the morphology of the PCVs attributable to this treatment could be observed. The same was true when systemic RACTS treatment was combined with local SRBC-immunization.

The present results give additional evidence to the concept on the structural and functional analogy between the chicken cloacal bursa and the mammalian GALT and lymph nodes. This suggests that the bursa can function like a peripheral lymphatic organ as well.

## INTRODUCTION

Recently, the chicken cloacal bursa (of Fabricius) has been shown to exert functions of a peripheral lymphatic organ (Naukkarinen, 1982), in addition to its functions as a central lymphatic organ providing a microenvironment for the differentiation and maturation of B lymphocytes (Toivanen and Toivanen, 1973, Toivanen et al., 1974). In mammals, the peripheral lymphatic organs always contain a distinct T cell area adjacent to the B cell regions, e.g. paracortex in the lymph nodes; interfollicular zone in the gut-associated lymphoid tissue (GALT). These T cell areas are characterized by post capillary venules (PCVs), normally lined by a high cuboidal endothelium (Gowans and Knight, 1964, Kruger, 1968, Cottier et al., 1973, Gutman and Weissman, 1973, Ford, 1975, Syrjänen, 1978, 1982). As previously shown in mammals, including man, these vessels are subject to structural changes related to the state of the immune response, for

example in the lymph nodes reacting to various antigenic stimuli (Krüger, 1968, Ford, 1975, Syrjänen, 1978, Kittas and Henry, 1981). A morphologically distinct T cell area, called diffusely infiltrated area (DIA) has recently been described in the chicken cloacal bursa as well (Odend'hal and Player, 1979, Odend'hal and Breazile, 1979, 1980). Analogous to the situation in mammals, it was shown that also the PCVs in the DIA 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).

When rendered T lymphocyte depleted with anti-theta or anti-thymus globulin treatment, mice show profound changes in the structure of the PCVs in the T cell areas of their lymphatic tissues (Cottier et al., 1973, Syrjänen, 1978, 1982). In chicken, the anti-thymus serum has been used mainly for characterization of the surface antigens on T lymphocytes (McArthur et al., 1971, Pink et al., 1981, Thunold et al., 1982), but no reports are available on its effects on the structure of the T cell areas after administration of T-dependent antigens.

In the present study, it is shown that chickens treated with rabbit anti chicken T lymphocyte serum (RACTS) show profound morphological changes in the bursal T cell area as determined morphometrically.

## 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 RACTS (rabbit anti chicken T lymphocyte serum)

Thymic lobes from 4-week-old chickens were removed and homogenized in Hank's balanced salt solution. The cells were washed and resuspended in phosphate buffered saline, and 1 ml of the suspension containing ca $10^8$ cells was injected into the ear vein 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°C) and adsorbed with chicken red blood cells and liver cells. The serum caused lysis at more than 90% of chicken thymic lymphocytes in a dilution of 1:64, but less than 10% of chicken bursal lymphocytes when tested undiluted, in an in vitro microcytotoxicity test with guinea pig complement. When applied to chickens RACTS was used undiluted.

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

### Application procedures

Twelve series of animals (five chickens in each) were studied, six of which (1A, 1B, 2A, 2B, 5A, 5B) served as controls. All the chickens were immunized with a 10% suspension of SRBC.

The chickens in Series 1A were given 200 µl RS per anum 6 hrs before per anum immunization with 200 µl 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 RACTS. The chickens in Series 2A

were given RS i.v. and those in Series 4A RACTS i.v. followed by per anum immunization with SRBC. In Series 5A and 6A, 200 µl SRBC was injected to chickens i.p. once at the start of the experiment. In Series 5A, the chickens were given 200 µl RS i.v. once a day for five consecutive days starting from the day of SRBC immunization. In Series 6A, RACTS was injected instead of RS.

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. The only exception was the dose of SRBC used in the i.p. immunization which was raised to 1 ml in the second injection in Series 5B and 6B.

## Sampling and measurements

In both series A and B, the animals were killed on the third day from the last antigen challenge with an overdose of an intravenous anaesthetic (Mebunat, Orion Oy, Espoo, Finland). The bursae were removed and their dorsal parts containing the DIA were frozen in isopentane cooled with liquid nitrogen. Cryostat sections, 4 µm thick, were stained with acid α-naphthyl acetate esterase (ANAE; Schlake et al., 1978) for cell counting, and formalin-fixed (10 min) and stained with hematoxylin and eosin for PCV measurements.

From an ANAE-stained section of each DIA studied, 1000 cells from every other field were systemically counted using an eye-piece graticule (E 34 A, Graticules Ldt., Tonbridge Kent, England) and a 40x objective in a Leitz Orthoplan light microscope. The cells displaying a dark brown spot-like cytoplasmic reaction product were considered T cells, whereas cells of the monocyte-macrophage series (MPS cells) stained diffusely brown throughout their cytoplasm. The cells remaining negative for ANAE included the cells present in the DIA other than T cells and MPS cells, i.e. B cells (Odend'hal and Player, 1979, Odend'hal and Breazile, 1980, Naukkarinen, 1982). The percentages of all these cells were calculated.

From each DIA studied, 10 PCVs were randomly selected and subjected to direct measurements using an eye-piece graticule (E 1, Graticules Ldt., Tonbridge Kent, England) and a 63x objective in a Leitz Orthoplan light microscope. The characteristics measured were: $D_{pcv}$, the diameter of the PCV; $D_{lu}$, the diameter of the PCV lumen; $H_{end}$, the height of the PCV endothelium; and MI, migration index (Kittas and Henry, 1981) thought to reflect the degree of lymphocyte recirculation. All parameters were measured as previously detailed (Syrjänen and Naukkarinen, 1982).

In comparison of the means, Student's t test was used where separately indicated.

## RESULTS

The effects of SRBC immunization, the RACTS treatment and their conbinations on the number of T cells, B cells and MPS cells in the DIA are summarized in Table 1. As expected, the number of T lymphocytes was very significantly ($p<0.001$) lowered by the RACTS treatment regardless of the application schedule used. The number of MPS cells was decreased in the RACTS treated and per anum stimulated chickens (Series 3A, 3B and 4A, 4B) when compared with their controls (1A, 1B and 2A, 2B, respectively). In the RACTS treated and i.p. stimulated chickens (Series 6A and 6B), on the other hand, the number of MPS cells showed a slight increase from the control values (Series 5A and 5B, respectively).

The parameters measured and those calculated in the PCVs of the DIA are summarized in Table 2. As evident, the per anum RACTS treatment

combined with subsequent per anum immunization with SRBC, had a prominent influence on the structure of the PCVs in the DIA when compared to the appropriate controls. These changes are exemplified by decrease in $D_{pcv}$ and $D_{lu}$ and flattening of $H_{end}$. The $H_{end}$ decreased from 4.33 ± 0.30 µm to 2.93 ± 0.36 µm ($p<0.01$) in Series 1A to 3A, and from 4.38 ± 0.23 µm to 3.16 ± 0.35 µm ($p<0.001$) in Series 1B to 3B. The RACTS, applied i.v. and followed by i.p. immunization with SRBC (Series 6A and 6B), had no significant effects on the PCV morphology, when compared to the controls (5A and 5B).

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

| SERIES | T CELLS (M±SEM) | B CELLS (M±SEM) | MPS CELLS (M±SEM) |
|---|---|---|---|
| 1A | 8.46 ± 0.81 | 90.22 ± 0.82 | 1.32 ± 0.13 |
| 1B | 9.44 ± 0.49 | 89.22 ± 0.64 | 1.34 ± 0.24 |
| 2A | 8.68 ± 0.31 | 89.10 ± 0.23 | 2.22 ± 0.35 |
| 2B | 8.28 ± 1.01 | 89.80 ± 0.99 | 1.92 ± 0.27 |
| 3A | 3.70 ± 0.38 | 96.02 ± 0.35 | 0.32 ± 0.13 |
| 3B | 4.50 ± 0.51 | 94.88 ± 0.61 | 0.62 ± 0.17 |
| 4A | 3.12 ± 0.27 | 96.28 ± 0.38 | 0.60 ± 0.15 |
| 4B | 2.52 ± 0.22 | 97.00 ± 0.25 | 0.60 ± 0.13 |
| 5A | 6.84 ± 0.38 | 92.54 ± 0.47 | 0.62 ± 0.10 |
| 5B | 6.10 ± 0.43 | 93.48 ± 0.46 | 0.42 ± 0.16 |
| 6A | 3.36 ± 0.20 | 96.00 ± 0.21 | 0.64 ± 0.12 |
| 6B | 3.38 ± 0.49 | 95.86 ± 0.69 | 0.76 ± 0.22 |

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

| SERIES | MI (M±SEM) | $D_{pcv}$, µm (M±SEM) | $D_{lu}$, µm (M±SEM) | $H_{end}$, µm (M±SEM) |
|---|---|---|---|---|
| 1A | 2.09 ± 0.21 | 18.14 ± 0.76 | 9.01 ± 0.29 | 4.33 ± 0.30 |
| 1B | 2.56 ± 0.21 | 21.42 ± 1.57 | 12.44 ± 1.04 | 4.38 ± 0.23 |
| 2A | 2.23 ± 0.38 | 17.35 ± 1.27 | 9.91 ± 1.27 | 3.73 ± 0.32 |
| 2B | 2.22 ± 0.22 | 15.75 ± 1.40 | 9.73 ± 1.41 | 3.15 ± 0.17 |
| 3A | 2.67 ± 0.20 | 14.69 ± 2.00 | 8.84 ± 0.49 | 2.93 ± 0.36 |
| 3B | 2.38 ± 0.28 | 16.33 ± 1.51 | 10.02 ± 1.11 | 3.16 ± 0.35 |
| 4A | 2.13 ± 0.24 | 17.68 ± 0.73 | 12.21 ± 0.97 | 2.74 ± 0.21 |
| 4B | 1.85 ± 0.19 | 17.25 ± 0.48 | 10.43 ± 0.64 | 3.41 ± 0.21 |
| 5A | 2.18 ± 0.21 | 20.10 ± 1.75 | 12.99 ± 1.20 | 3.56 ± 0.37 |
| 5B | 2.19 ± 0.21 | 18.43 ± 0.68 | 12.79 ± 0.57 | 2.82 ± 0.14 |
| 6A | 2.65 ± 0.09 | 18.32 ± 0.80 | 11.38 ± 0.68 | 3.47 ± 0.30 |
| 6B | 2.22 ± 0.15 | 18.52 ± 1.31 | 12.10 ± 1.45 | 3.21 ± 0.38 |

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

## DISCUSSION

In chicken, anti T lymphocyte serum has been used mainly to detect the T cell surface antigens (McArthur et al., 1971, Pink et al., 1981, Thunold et al., 1982). Studies with anti T lymphocyte sera have further revealed that only very small amounts of T lymphocytes reside in the chicken bursa as determined from the bursal cell suspensions (Potworowski, 1972, Albini and Wick, 1974). With ANAE staining on sections, however, Odend'hal and coworkers succeeded in finding the diffusely infiltrated area (DIA) in the bursa. According to their studies, T cells comprise 18.8% of the cells in the DIA (Odend'hal and Breazile, 1979, 1980, Odend'hal and Player, 1979). In the present work, the percentages of T cells remained around 10% even in chickens treated locally with RS and SRBC for two weeks (Table 1, Series 1B). This discrepancy could simply be due to a different section thickness, which was reported 8-10 µm by Odend'hal and Breazile (1980), and 4 µm in this study.

More important than the absolute number of cells in the DIA, however, were the changes in the number of T cells caused by the RACTS treatment. The most striking effect was achieved when RACTS was given i.v. and combined with subsequent per anum immunization with SRBC for two weeks (Series 4B); the percentage of T cells in the DIA decreased very significantly ($p<0.001$). The same result was obtained when both RACTS and SRBC were applied per anum for two weeks (Series 3B). The combined systemic administration of RACTS (i.v.) and SRBC (i.p.) had also a clearcut local effect; the number of T cells in the DIA was decreased to half (Table 1).

The number of MPS cells decreased, though to a smaller extent, along with the T cells in the DIA due to the RACTS treatment. In the DIA of chickens treated systemically with RACTS and SRBC (Series 6A and 6B) the number of macrophages showed a slight increase. This might be due to the dual function of macrophages; besides collaborating T cells in the immune response they phagocytose the cells destroyed by RACTS. The function of the MPS cells in the DIA will be further analyzed in the following work where a specific antiserum against them is used.

The morphometrical measurements of the PCVs in the DIA gave further support to our previously presented idea that they undergo structural changes due to local immunization with SRBC (Syrjänen and Naukkarinen, 1982). Whereas the per anum stimulation with SRBC induces hypertrophy of the PCV endothelium the RACTS treatment depletes T cells in the DIA and causes atrophy and flattening of the PCV endothelium. Correspondingly, changes of the PCVs into either hypertrophic or atrophic directions depending on the current state of lymphocyte circulation and immunological reactivity have been reported to occur in the mammalian T cell regions as well (Kruger, 1968, Ford, 1975, Syrjänen, 1978, 1982, Kittas and Henry, 1981). The dilatation of the PCV lumen observed in all the chickens treated with RACTS is solely attributable to the lowered endothelial height ($H_{end}$), since the diameter of these venules ($D_{pcv}$) was also markedly decreased (Table 2). The migration index, MI (Kittas and Henry, 1981) did not significantly change due to the RACTS treatment. This is a seemingly controversial finding to that of our previous work (Syrjänen and Naukkarinen, 1982), but the atrophic PCV endothelium could well be so damaged by RACTS that passage of lymphocytes through the endothelium was inhibited thus increasing the value of MI, as has been shown to occur in mammals (Syrjänen, 1982). This could also explain why even in chickens affected most severely by RACTS (Series 4B) the MI did not significantly differ from the control value. In this respect, noteworthy may be the finding that RACTS exerts hardly any effects on the PCVs of the DIA in systemically

immunized chickens. This means that the PCVs in the DIA are subject to structural changes caused by local immunization only, and agrees with the previously presented reports on mammals (Kruger, 1968, Kittas and Henry, 1981).

The present study further indicates that the bursal DIA in many respects is structurally and functionally analogous to the T cell regions of the mammalian lymphatic tissues, the lymph nodes and GALT in particular (Odend'hal and Breazile, 1079, 1980, Odend'hal and Player, 1979, Naukkarinen, 1982, Syrjänen and Naukkarinen, 1982). This is a strong argument in favour of the concept that the chicken cloacal bursa also has functions of a peripheral lymphatic organ.

REFERENCES

Albini B and Wick G. Delineation of T and B lymphoid cells in the chicken. J Immunol 1974: 112: 445-450.

Cottier H, Turk J and Sobin L. A proposal for standardized system of reporting human lymph node morphology in relation to immunological function. J Clin Path 1973: 26: 317-331.

Ford WL. Lymphocyte migration and immune responses. Prog Allergy 1975: 19: 1-59.

Gowans JL and Knight EJ. The route of recirculation of lymphocytes in the rat. Proc Roy Soc Biol 1964: 159: 257-282.

Gutman GA and Weissman IL. Homing properties of thymus-independent follicular lymphocytes. Transplantation 1973: 16: 621-629.

Kittas C and Henry L. BCG-induced changes in the post capillary venules of the guinea pig lymph node. Lymphology 1981: 14: 24-28.

Kruger G. Morphology of postcapillary venules under different experimental conditions. J Natn Cancer Inst 1968: 41: 287-301.

McArthur WP, Chapman J and Thorbecke GJ. Immunocompetent cells of the chicken. I. Specific surface antigenic markers on bursa and thymus cells. J Exp Med 1971: 134: 1036-1045.

Naukkarinen A. The chicken bursa of Fabricius as a peripheral lymphoid organ. A light and electron microscopic study on the transport of colloidal carbon in the bursa. Ph.D. Thesis, Department of Pathology, University of Kuopio, Kuopio, Finland, 1982.

Odend'hal S and Breazile JE. Diffusely infiltrated area of lymphoid cells in the cloacal bursa. J Reticuloendothel Soc 1979: 25: 315-324.

Odend'hal S and Breazile JE. An area of T cell localization in the cloacal bursa of White Leghorn chickens. Am J Vet Res 1980: 41: 255-258.

Odend'hal S and Player EC. Histochemical localization of T cells in tissue sections. Avian Dis 1979: 24: 886-895.

Pink JRL, Fedecka-Bruner B, Coltey M, Péault BM and Le Douarin NM. Biochemical characterization of avian T lymphocyte specific antigens. Eur J Immunol 1981: 11: 517-520.

Potworowski EF. T and B lymphocytes. Organ and age distribution in the chicken. Immunology 1972: 23: 199-204.

Schlake W, Meyer EM and Grundmann E. Histochemical identification of T and B areas in paraffin-embedded lymphoid tissues by demonstration of α-naphthyl acetate esterase (ANAE) activity. Path Res Pract 1978: 163: 173-182.

Sorvari T, Sorvari R, Ruotsalainen P, Toivanen A and Toivanen P. Uptake of environmental antigens by the bursa of Fabricius. Nature (Lond.) 1975: 253: 217-219.

Syrjänen KJ. Post-capillary venules of the lymphatic tissues in mice with special reference to the depletion of T-lymphocyte population. Exp Molec Pathol 1978: 29: 291-302.

Syrjänen KJ. The lymph nodes. Reactions to experimental and human tumors. Exp Pathol 1982: Suppl 8: 1-123.

Syrjänen KJ and Naukkarinen A. Post-capillary venules in the T-cell area of chicken cloacal bursa with reference to the state of local immune response. Morphometry in Morphological Diagnosis, Y Collan and T Romppanen (editors), Kuopio University Press, Kuopio 1982: 211-216.

Thunold S, Boyd R, Schauenstein K and Wick G. Tissue localization of lymphocyte surface antigens and receptors for immunoglobulin G Fc and complement in the chicken. J Histochem Cytochem 1982: 30: 201-206.

Toivanen P and Toivanen A. Bursal and post-bursal stem cells in chicken. Functional characteristics. Eur J Immunol 1973: 3: 585-595.

Toivanen P, Toivanen A and Tamminen P. Bursal and post-bursal cells in chicken. Occurrence of postbursal cells in bone marrow, thymus and spleen. Eur J Immunol 1974: 4: 405-410.