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Ontogeny of T cells, B cells and monocytes in the bovine foetus

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SUMMARY

The ontogeny of lymphocyte subpopulations and monocytes was studied in developing bovine foetuses. Fifty-four bovine foetuses ranging in age from 3 to 9 months of gestation were collected and examined for the presence of thymus-derived (T lymphocytes), bone marrow-derived (B lymphocytes) and phagocytic (macrophage) cells from thymus, spleen and peripheral blood. Detection of the cells expressing T lymphocyte surface markers was done using a modified sheep red blood cell rosette assay. Lymphocytes bearing surface immunoglobulin (sIg) were detected by staining with a fluoresceinated rabbit antibody prepared against bovine IgG. The procedure used for detecting monocytes was a latex bead phagocytic assay. In the thymus, the percentage of T lymphocytes was relatively constant throughout gestation at approximately 60–70%, with B lymphocytes remaining at about 1%, whereas the phagocytic cells showed an increase from 1% at 3 months to approximately 8% at term. The spleen showed an increasing percentage of T lymphocytes, from 11% at 3 months to over 40% at term, whereas the percentage of B lymphocytes remained consistent at about 2–3% from 3 months until term. The percentage of monocytes increases from 1% at 3 months to over 20% at 9 months. Peripheral blood T lymphocytes increased steadily from approximately 1% at 3 months to 45% at term. B lymphocyte levels remained low, about 1%, throughout gestation, whereas monocytes increased from 1% at 3 months to 5% at 9 months.

INTRODUCTION

During ontogenic development, all cell types involved in responses to antigenic stimulation differentiate. Little is known about the sequence of maturation of these various cell types, e.g. T, B and macrophage in cattle. The purpose of the current study was to study the ontogeny of lymphocytes and monocytes in the foetal bovine thymus, spleen and peripheral blood.

MATERIALS AND METHODS

Source of foetus. Fifty-four bovine foetuses ranging in age from 3 to 9 months were obtained from a local abattoir. Foetal ages were estimated from crown to rump measurements (Roberts, 1971).

Lymphocyte suspensions. Peripheral foetal blood was obtained by cardiac puncture and placed into tubes containing heparin (50 u/ml blood). Lymphocytes were separated by the Ficoll–Hypaque technique (Böyum, 1968). Briefly, heparinized blood was layered on to Ficoll–Hypaque (sp. gr. 1·077) and centrifuged at 400 *g* at 20°C for 30 min. The interface was carefully aspirated and washed three times with Hanks' balanced salt solution (HBSS). The cell preparation contained approximately 95% mononuclear cells. Approximately 95–98% of the cells were judged viable by the exclusion of trypan blue dye.

Splenic lymphocytes and thymocyte suspensions were obtained by mincing the tissue with scissors after removing extraneous connective tissue. HBSS was added to the petri dishes and cell suspensions were transferred to 50 ml conical tubes.

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After the larger particles had settled, the cell-rich supernatant was aspirated on to Ficoll–Hypaque solution. Samples were centrifuged at 400 *g* for 30 min at which time the interface was removed, washed three times with HBSS and examined for T and B cell content.

Marker for monocytes. Monocytes were identified by their ability to ingest latex beads. Cell preparations were adjusted to approximately 2×10^6 cells/ml in HBSS, and 1·0 ml of a 1:50 dilution of 0·81 μ diameter latex particles (Difco, Detroit, Michigan) was added to a 10 ml cell suspension. This mixture was incubated at 37°C for 1 hr with continuous low speed rotation (Multipurpose rotator, Scientific Industries, Inc., Springfield, Maryland). After the incubation, the cells were washed three times with 50 ml of HBSS. These cells were then stained for sIg or assayed for rosette formation. Cells ingesting three or more latex beads were considered positive and referred to as monocytes.

Quantification of surface Ig-bearing cells. Surface membrane immunoglobulin (sIg) bearing cells were identified by methods described previously (Muscoplat *et al.*, 1974; Paul *et al.*, 1977; Senogles *et al.*, 1978). Briefly, a cell suspension containing 10^6 cells was added to small conical centrifuge tubes (Analytic Lab Accessories, West Hempstead, New York) and washed three times in phosphate buffered saline (PBS) containing 0·2% sodium azide and 1% bovine serum albumin (BSA), and then pelleted by centrifugation at 800 *g* for 2 min. The pellets were resuspended in 50 μl of a 1:10 dilution of a fluoresceinated rabbit anti-bovine IgG* (heavy and light chains, Miles Laboratories, Elkhart, Indiana), vortex mixed and incubated at 4°C for 30 min. After incubation, the cells were washed three times in PBS with sodium azide and BSA. The cell preparations were then examined by alternating phase and fluorescent microscopy. The percentage of cells bearing SIG was determined by counting a minimum of 200 cells. Cells ingesting latex beads were excluded from the calculation of sIg-bearing cells.

Marker for T lymphocytes. The percentage of lymphocytes bearing cell surface markers for T lymphocytes were identified by a previously described rosetting method (Paul *et al.*, 1978).

Lymphocytes (100 μl), resuspended in rosetting medium† at 10^7 cells/ml, were added to 200 μl of a 1% sheep red blood cell (SPBC) suspension in small conical, 1·0 ml centrifuge tubes and then incubated in a 37°C water bath for 10 min. The cells were then centrifuged at 200 *g* for 5 min at room temperature. After overnight incubation at 4°C, a drop of 0·5% trypan blue dye was added. The cells were resuspended by gently rocking the tube lightly until the cells were homogenously dispensed. A drop of the suspension was placed in a haemocytometer and 200 live cells were counted. A lymphocyte with three or more adherent SRBC was defined as a rosette-forming cell (RFC).

Statistical analysis. Methods described by Colton (1974), were followed for regression analysis.

RESULTS

Frequency of rosette-forming cells in the thymus spleen and peripheral blood of the developing bovine foetus

The frequency of T cells that appear in the developing bovine foetus, ranging in age from 3 months to 9 months, was studied. Fig. 1 shows the percentage of RFC that occur during bovine foetal development. The percentage of RFC found in the thymus remained relatively constant at between 60 and 70% from 3 months to term. Both the spleen and peripheral blood show a marked increase in T cells from 3 to 9 months, with the spleen increasing from about 11–41% and the numbers in the peripheral blood increasing from 0–45%, respectively.

Frequency of sIg-bearing cells in the thymus, spleen and peripheral blood of the developing bovine foetus

The frequency of B cells that appear in the developing bovine foetus, ranging in age from 3 months to 9 months, was studied. Fig. 2 shows the percentages of B cells that occur during bovine foetal development. The spleen showed a constant level of 2·5% from 3 months to term, with the thymus and peripheral blood both maintaining a 1% level throughout foetal development.

Frequency of latex bead ingesting cells in the thymus, spleen and peripheral blood of the developing foetus

Fig. 3 shows the percentages of latex bead-ingesting cells that occur while the bovine foetus is undergoing development. The spleen shows 0% at 3 months, but rapidly increases to over 20% by 9 months. In the thymus, the phagocytic cells that ingest latex beads increase from approximately 1·5% at 3 months to 8% at term, with the peripheral blood showing a smaller increase from 0% at 3 months to 5% at term.

* Anti-IgG contains antibodies to both heavy and light chains of IgG. Studies in our laboratory have shown that this reagent also detects cells bearing IgM. Therefore, use of this reagent will detect both IgM- and IgG-bearing cells.

† *Rosetting medium.* Foetal calf serum (FCS) was heat-inactivated and absorbed with SRBC. One volume of FCS was mixed with 0·2 volumes of packed SRBC and incubated in a 37°C water bath for 1 hr. The FCS-SRBC mixture was further incubated for 1 hr at 4°C then centrifuged at 400 *g* for 20 min, and then the FCS was carefully aspirated. RPMI-1640 (Biolabs, Inc., Chicago, Illinois) tissue culture media containing 25 mM of HEPES buffer, containing 10% absorbed FCS, was used as the rosetting medium throughout this study.

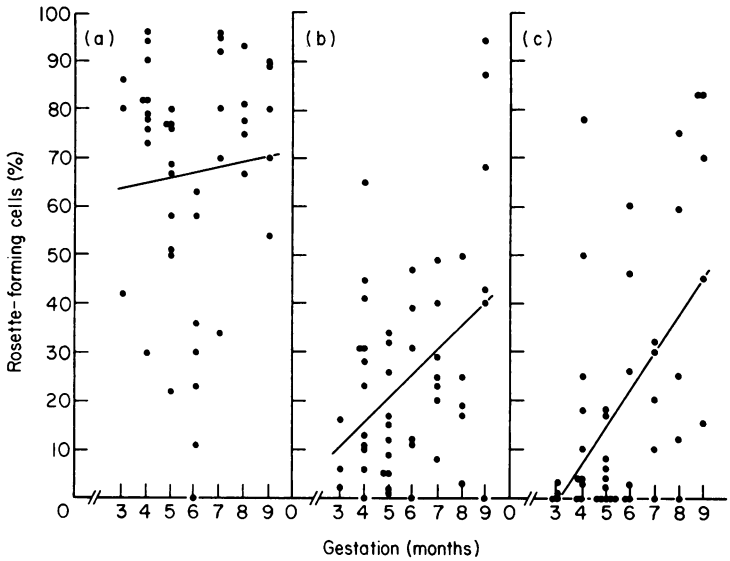

FIG. 1. The percentage of T lymphocytes found (a) in the thymus, (b) spleen and (c) peripheral blood of the developing bovine foetus. T lymphocytes were measured by a modified sheep red blood cell rosette forming assay. Scattergrams were fitted to a straight line by the method of linear regression analysis.

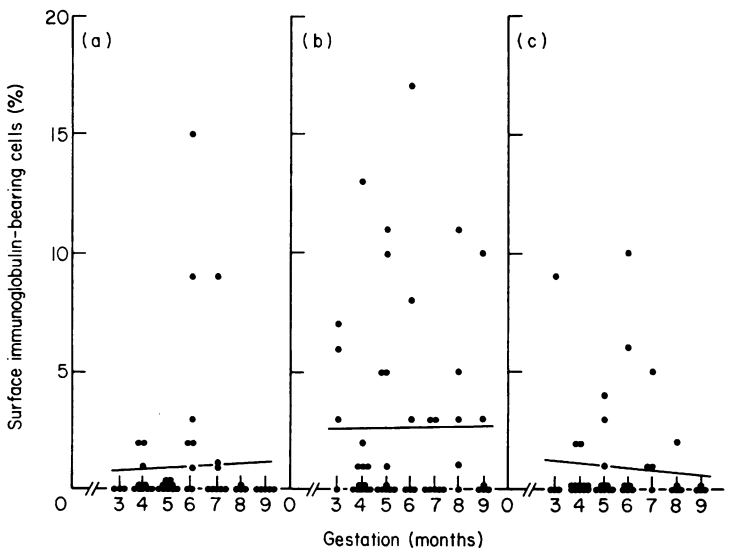

FIG. 2. The percentage of B lymphocytes found (a) in the thymus, (b) spleen and (c) peripheral blood of the developing bovine foetus. B lymphocytes were measured by staining for surface membrane immunoglobulin. Scattergrams were fitted to a straight line by the method of linear regression analysis.

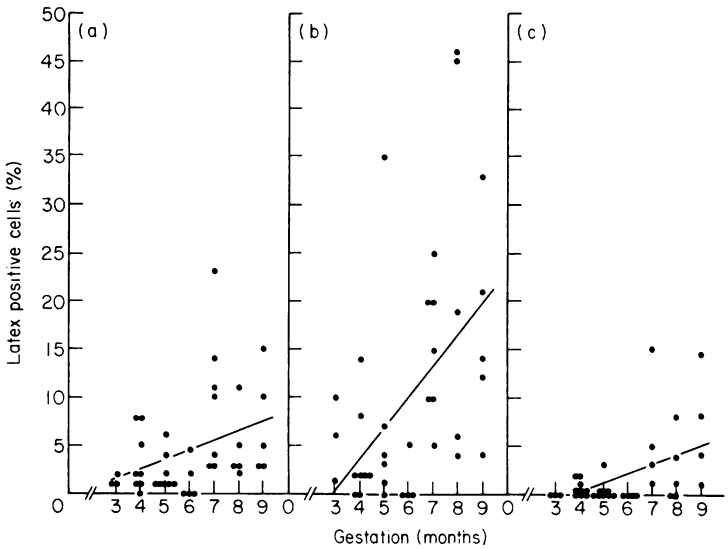

FIG. 3. The percentage monocytes found (a) in the thymus (b) spleen and (c) peripheral blood of the developing bovine foetus. Monocytes were measured by a latex bead ingestion assay. Scattergrams were fitted to a straight line by the method of linear regression analysis.

DISCUSSION

The present study has looked at the time at which T lymphocytes, B lymphocytes and monocytes appear during the development of the bovine foetus. T lymphocytes were present in large numbers as early as 3 months in the thymus, whereas they appeared at a lower percentage (11%) in the spleen at 3 months and were not present at all at 3 months in peripheral blood.

B lymphocytes were present in all three tissues at 3 months, with the thymus and peripheral blood showing a low percentage (1%) throughout gestation, whereas the spleen had a slightly higher percentage (2·5%) throughout gestation. Monocytes were present as early as 3 months in the thymus and spleen, but not the peripheral blood. Monocytes first appeared around the fourth month of gestation in the peripheral blood.

The percentages of T lymphocytes in the foetal thymus seems to indicate that the thymus is the primary organ responsible for seeding the other internal organs, since from 3 to 9 months the percentages gradually increased. The percentage of T lymphocytes found in the peripheral blood and spleen also indicates that more T lymphocytes are released from the thymus and increasing foetal age until term, when antigenic stimulation causes a further increase to reach the adult levels of approximately 63% for peripheral blood and 60% for spleen (Paul *et al.*, 1978).

The low percentages of antibody-producing B lymphocytes found in the foetal thymus, spleen and peripheral blood were as expected, since B lymphocyte differentiation and proliferation are mostly antigen-dependent (Cooper, Peterson & Good, 1970; Kearney & Lawton, 1975a, b; Sidman & Unanue, 1975; Olson & Waxler, 1977). However, it has been shown that some clones of specific antigen responsive cells can be found during development regardless of antigen exposure (Chapman, Johnson & Cooper, 1974; Melchers, Anderson & Phillips, 1976). The percentages of B lymphocytes found would also appear to be correct since the bovine placenta possesses an impermeable epitheliochorial membrane, which is very capable of preventing exposure to foreign antigens from the external environment. Other studies in our laboratory have shown that normal adult bovine B lymphocyte levels of peripheral blood are not reached until approximately 1 to 2 months of life and are about 20% (Senogles *et al.*, 1978).

Phagocytic cells (macrophages) originating from the bone marrow during foetal development require certain factors in order to differentiate into mature macrophages capable of exhibiting phagocytosis

(Guez & Sachs, 1973). The site of differentiation of macrophages is unknown, but it has been shown that factors released by immunocompetent T lymphocytes or mature macrophages are needed (Hardy, Globerson & Danon, 1973). The percentage of macrophages (latex-ingesting cells) was the highest in the foetal spleen, which started with 11% at 3 months and had an increasing number of T lymphocytes present, 41% at 9 months. Very few phagocytic cells were found in the thymus or peripheral blood even though both possessed a concentration of T lymphocytes greater than that found in the spleen. This may indicate that the foetal spleen could possibly, along with the bone marrow, be a site of macrophage differentiation in the bovine foetus.

In summary, we have shown the ontogeny of T and B lymphocytes, as well as macrophages in the thymus, spleen and peripheral blood of the developing bovine foetus. We feel that this new knowledge may help us to understand better the functioning of the bovine immune system and the relationships between development of T and B lymphocytes and their cooperation in immune responses.

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