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The development of intraepithelial and Peyer's patch lymphocyte sub-types in the small intestine of newborn rats

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SUMMARY

Small intestinal intraepithelial (IEL) and Peyer's patch (PPL) lymphocytes of newborn rats have been studied in histological sections and in isolated cell suspensions. Initially IEL numbers were low compared with older animals, and fewer cells were labelled by the monoclonal antibody markers used. At birth, 41% of IEL expressed receptors for MRC OX8 (T suppressor marker) yet lacked receptors for W3/13 (pan-T marker) which was present on only 1% of these cells. IEL with receptors for W3/25 (T helper marker) were not seen until 2 weeks of age. Granulated cells (GIEL) accounted for only 19% of IEL at birth. The numbers and relative proportions of these lymphocyte sub-types were still not mature at the time of weaning. In the first week of life there was a short lived upsurge in numbers of GIEL and MRC OX8$^+$ IEL. The relative distribution of PPL sub-types was mature 4 weeks after birth, but the morphology of this tissue did not appear to be well differentiated until 1 week later.

Keywords mucosal lymphocytes rat neonates development Peyer's patches intraepithelial lymphocytes

INTRODUCTION

In the neonate and infant there is evidence that the gastrointestinal immune system is functionally immature. Savilahti (1972) showed that the quantitative development of IgA production in the gut mucosa is not fully established in man until 2 years of age. Clinically in the infant there is an increased susceptibility to infections and hypersensitivity reactions, particularly in non-breast fed babies (Bullen & Willis, 1971; Matthew *et al.*, 1977). At birth, Peyer's patches are histologically rudimentary in structure (Ferguson & Parrott, 1972; Joel, Hess & Cottier, 1972) and intraepithelial lymphocyte (IEL) numbers are reduced (Orlic *et al.*, 1981; Thomas & Anderson, 1982). We have previously identified lymphocyte subsets amongst Peyer's patch lymphocytes (PPL) and IEL, which express markers associated with T suppressor and helper function, using isolated rat mucosal lymphocyte preparations (Lyscom & Brueton, 1982). In this study we have investigated the possibility that in the newborn there may be an imbalance of helper and suppressor cells by studying the distribution of IEL subtypes in neonatal rat gut from birth into the post-weaning period.

MATERIALS AND METHODS

Animals. Category 3 inbred PVG rats (PVG/Ola) obtained initially from Olac Limited, Bicester, were bred in the Westminster Medical School animal house. The young were weaned at 4

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weeks of age. Three to five groups of animals from different litters were sacrificed during the first 24 h after birth and at weekly intervals thereafter.

Preparation of intraepithelial and Peyer's patch lymphocytes, immunofluorescence and cytochemical staining. These were described in detail in a previous publication (Lyscom & Brueton, 1982). Briefly, the small intestine was flushed through thoroughly with citrate buffer to remove the gut contents, the Peyer's patches were removed, and IEL were released by incubation of the tissues in the same buffer followed by vortex agitation. Separation of the epithelium from the lamina propria was confirmed by histological examination. Cells were released from the Peyer's patches by scraping with a scalpel. The cell preparations were purified by filtration through cotton gauze and cotton wool and by density gradient centrifugation using Lymphoprep (Nyegaard and Company, A/S, Oslo). B cells with surface immunoglobulins were assayed using fluorescein conjugated anti-rat immunoglobulin (Wellcome Reagents Limited, London). Cells expressing T cell markers were labelled by indirect assays using the monoclonal antibodies W3/13 (pan-T), W3/25 (T helper) and MRC OX8 (T suppressor) (Seralab Limited, Sussex). Fluorescein conjugated anti-mouse IgG (Miles Laboratories Limited, Slough) was used as the second antibody. Preparations were studied under u.v. light. Cells were also examined in Giemsa/May Grunwald stained cytocentrifuge preparations. IEL numbers per 500 epithelial cells and tissue morphology were determined on histological sections. Serial sections were made in order to identify Peyer's patches from the young animals. These preparations were stained with Harris' haematoxylin and 1% eosin.

Statistical analyses. IEL and granulated IEL (GIEL) numbers per 100 epithelial cells were compared using two-tailed unpaired *t*-tests. The Kriskal–Wallis test and the Mann–Whitney U-test were used to compare percentage values of GIEL and MRC OX8$^+$ IEL.

RESULTS

Observations at birth

IEL were present in low numbers at birth (3·4/100 epithelial cells). In stained cytocentrifuge preparations the cytoplasm of IEL was bluer than in older animals. Blast cells and cells in mitosis were not observed and were rarely seen amongst IEL at any time. GIEL accounted for 19% of the cells on the first day of life. At this time most IEL were negative to the markers applied, 41% expressed the MRC OX8 marker but lacked receptors for W3/13 which was present on only 1% of the cells. W3/25$^+$ IEL were not evident at birth, neither were B cells which were also absent from

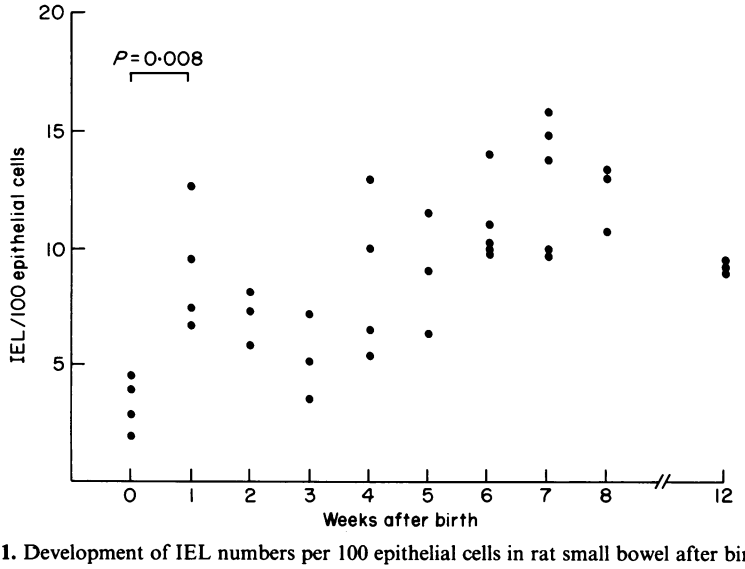

Fig. 1. Development of IEL numbers per 100 epithelial cells in rat small bowel after birth.

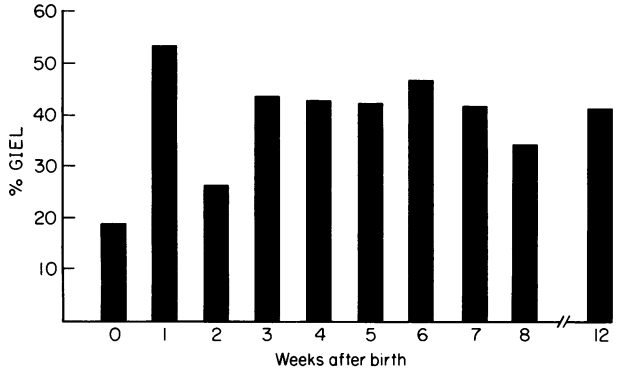

Fig. 2. Percentages of granulated IEL in rat small bowel at birth and at weekly intervals thereafter, values represent means.

mature IEL. Recognizable Peyer's patches were poorly structured at birth although regions of aggregated lymphocytes could be seen on histological sections; germinal centres were absent.

Development of intraepithelial lymphocytes

The development of IEL number can be seen in Fig. 1. They increased significantly during the first week of life ($P = 0{\cdot}008$) and fell over the next 2 weeks, although not significantly, before rising again to reach mature values approximately 6 weeks after birth. The percentages of GIEL followed a similar pattern (Fig. 2) and their numbers expressed per 100 epithelial cells increased ($P = 0{\cdot}0036$) and decreased ($P = 0{\cdot}0208$) significantly during this time. Fig. 3 shows the mean percentages of IEL subtypes at birth and at weekly intervals thereafter. The proportion of MRC OX8$^+$ IEL also appeared to peak 1 week after birth. Although this did not reach statistical significance, their numbers per 100 epithelial cells, derived by calculation, did increase before ($P = 0{\cdot}001$) and decrease after ($P = 0{\cdot}015$) this time. W3/25$^+$ IEL were first detected 2 weeks after birth. The numbers of these cells and of W3/13$^+$ IEL increased gradually reaching maturity by 6 weeks of age: MRC OX8$^+$ IEL took longer to develop.

Peyer's patch lymphocytes

It was not possible to obtain PPL uncontaminated by other mucosal lymphocytes prior to 4 weeks

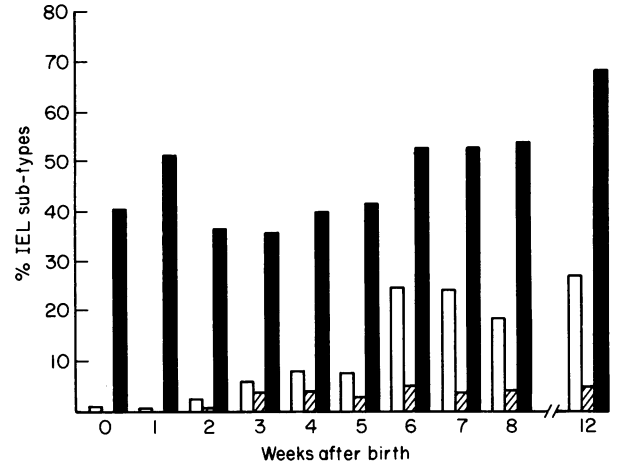

Fig. 3. Percentages of IEL sub-types in rat small bowel at birth and at weekly intervals thereafter, values represent means. □ = W3/13; ■ = W3/25; ■ = MRC OX8.

after birth. At this time and during the subsequent weeks the distribution of lymphocyte subtypes did not differ markedly from the values obtained from animals over 8 weeks of age.

DISCUSSION

This study has shown that IEL numbers and their sub-type distribution are immature in neonatal rats. This coincides with a period in mice in which tolerance mechanisms to oral antigen are impaired (Hanson, 1981). Although the role of IEL is uncertain they have been implicated in animal studies, with delayed type hypersensitivity, cell-mediated lympholysis (CML), natural killer (NK) activity and antibody-dependent cytotoxicity (Arnaud-Battandier *et al.*, 1978; Mowat & Ferguson, 1982; Davies & Parrott, 1981; Tagliabue *et al.*, 1981). Recent investigations in mice by the authors suggest that they may enhance local mucosal antibody responses.

Peyer's patches were poorly structured in the first 4 weeks of life, but there was a mature distribution of lymphocyte subtypes by the time of weaning. The importance of PPL subtypes in the regulation of mucosal immune responses has been shown by their role in the enhancement of IgA and in the suppression of IgG and IgE production following exposure to oral antigen (Ngan & Kind, 1978; Richman *et al.*, 1982).

The MRC $OX8^+$, $W3/13^-$ population of cells which was prominent at birth was found to predominate amongst mature IEL in a previous study (Lyscom & Brueton, 1982). Comparable cells taking up the Lyt-2 and OKT8 but not all pan-T markers have been found in mice and man respectively (Davies & Parrott, personal communication; Selby *et al.*, 1982). Their role is uncertain. However MRC $OX8^+$ lymphocytes from other sources have been shown to function as suppressor cells and NK cells (Brideau *et al.*, 1980; Cantrell *et al.*, 1982), and it can be inferred from the experiments of Loop, Bernstein & Wright (1980) and those of Brideau *et al.* (1980) that the MRC OX8 antibody also labels mixed lymphocyte reaction (MLR) induced cytotoxic T cells. GIEL were also present at birth; they are considered to express NK activity (Tagliabue *et al.*, 1982).

The short lived but significant increase in GIEL and MRC $OX8^+$ IEL numbers during the first week of life is a particularly interesting finding. This is unlikely to reflect an immune response against milk components since it did not continue throughout the suckling period. It is improbable that it represents NK cell activity or CML since, in suckling rodents these functions are late to develop (Brooks & Flannery, 1980; Pilarski, 1977). It is also unlikely to reflect an MLR type response since the cells lack the characteristic W3/25 marker (Webb, Mason & Williams, 1979; Loop *et al.*, 1980). The possibilities exist that the IEL seen soon after birth are either of maternal origin or are under the influence of milk lymphocytes. It has been shown in rats that lymphocytes of the maternal genotype have the potential to cross the mucosal barrier of F_1 hybrid offspring (Seelig & Billingham, 1981). The role of the non-B lymphocytes of colostrum and milk, which in man account for approximately 50% of the lymphocytes at birth (Diaz-Jouanen & Williams, 1974) is not well understood. However, an association between these cells and IEL is indicated by their surface marker characteristics (Richie *et al.*, 1982) and from their common site of origin (Rose, Parrott & Bruce, 1978; Manning & Parmley, 1980; Guy-Grand, Griscelli & Vassalli, 1974).

Further studies of the nature and functions of both IEL and milk lymphocytes are required to increase our understanding of the neonatal gut mucosal mechanisms involved in relation to the development of immunoregulation and cell-mediated immunity.

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REFERENCES

ARNAUD-BATTANDIER, F., BUNDY, B.M., O'NEILL, M., BIENENSTOCK, J. & NELSON, D.C. (1978) Cytotoxic activity of gut mucosal lymphoid cells in guinea-pigs. *J. Immunol.* **121,** 1059.

BRIDEAU, R.J., CARTER, P.B., MCMASTER, W.R., MASON, D.W. & WILLIAMS, A.F. (1980) Two subsets of rat T lymphocytes defined with monoclonal antibodies. *Eur. J. Immunol.* **10,** 609.

BROOKS, C.G. & FLANNERY, G.R. (1980) Quantitative studies of natural immunity to solid tumours in

rats. Persistance of natural immunity throughout reproductive life and absence of suppressor cells in infant rats. *Immunology*, **39,** 187.

BULLEN, C.L. & WILLIS, A.T. (1971) Resistance of the breast-fed infant to gastroenteritis. *Br. Med. J.* **3,** 338.

CANTRELL, D.A., ROBINS, R.A., BROOKS, C.G. & BALDWIN, R.W. (1982) Phenotype of rat natural killer cells defined by monoclonal antibodies marking rat lymphocyte subsets. *Immunology*, **45,** 97.

DAVIES, M.D.J. & PARROTT, D.M.V. (1980) The early appearance of specific cytotoxic T cells in murine gut mucosa. *Clin. exp. Immunol.* **42,** 273.

DIAZ-JOUANEN, E. & WILLIAMS, R.C. (1974) T and B lymphocytes in human colostrum. *Clin. Immunol. Immunopathol.* **3,** 248.

FERGUSON, A. & PARROTT, D.M.V. (1972) The effect of antigen deprivation on thymus-dependent and thymus-independent lymphocytes in the small intestine of the mouse. *Clin. exp. Immunol.* **12,** 477.

GUY-GRAND, D., GRISCELLI, C. & VASSALLI, P. (1974) The gut-associated lymphoid system: nature and properties of the large dividing cells. *Eur. J. Immunol.* **4,** 435.

HANSON, D.G. (1981) Ontogeny of orally induced tolerance to soluble proteins in mice. I. Priming and tolerance in newborns. *J. Immunol.* **127,** 1518.

JOEL, D.D., HESS, M.W. & COTTIER, H. (1972) Magnitude and pattern of thymic lymphocyte migration in neonatal mice. *J. exp. Med.* **35,** 907.

LOOP, S.M., BERNSTEIN, I.D. & WRIGHT, P.W. (1980) T cell synergy in the rat: serological characterisation of T cell subsets. *J. Immunol.* **125,** 1237.

LYSCOM, N. & BRUETON, M.J. (1982) Intraepithelial, lamina propria and Peyer's patch lymphocytes of the rat small intestine: isolation and characterization in terms of immunoglobulin markers and receptors for monoclonal antibodies. *Immunology*, **45,** 775.

MANNING, L.S. & PARMLEY, M.J. (1980) Cellular determinants of mammary cell-mediated immunity in the rat. I. The migration of radioisotopically labelled T lymphocytes. *J. Immunol.* **125,** 2508.

MATTHEW, D.J., TAYLOR, B., NORMAN, A.P., TURNER, M.W. & SOOTHILL, J.F. (1977) Prevention of eczema. *Lancet*, **i,** 321.

MOWAT, A. & FERGUSON, A. (1982) Intraepithelial lymphocyte count and crypt hyperplasia measure the mucosal component of the graft-versus-host reaction in mouse small intestine. *Gastroenterology*, **83,** 417.

NGAN, J. & KIND, L.S. (1978) Suppressor T cells for IgE and IgG in Peyer's patches of mice made tolerant by the oral administration of ovalbumin. *J. Immunol.* **120,** 861.

ORLIC, D., GRAY, B.A., CARMICHAEL, R.D. & BARON, P. (1981) Lymphocyte infiltration of gut epithelium in fetal and neonatal rabbits. *Biol. Neonate*, **40,** 91.

PILARSKI, L.M. (1977) Ontogeny of cell-mediated immunity. I. Early appearance of alloantigen-specific cytotoxic T-cell precursors in postnatal mice. *J. exp. Med.* **146,** 887.

RICHIE, E.R., BASS, R., MEISTRICH, M.L. & DENNISON, D.K. (1982) Distribution of T lymphocyte subsets in human colostrum. *J. Immunol.* **129,** 1116.

RICHMAN, L.K., GRAEFF, A.S., YARCHOAN, R. & STROBER, W. (1981) Simultaneous induction of antigen-specific IgA helper T cells and IgG suppressor T cells in the murine Peyer's patch after protein feeding. *J. Immunol.* **126,** 2079.

ROSE, M.L., PARROTT, D.M.V. & BRUCE, R.G. (1978) The accumulation of immunoblasts in extra vascular tissues including mammary gland, peritoneal cavity, gut and skin. *Immunology*, **35,** 415.

SAVILAHTI, E. (1972) Immunoglobulin containing cells in the intestinal mucosa and immunoglobulins in the intestinal juice in children. *Clin. exp. Immunol.* **11,** 415.

SEELIG, L.L. & BILLINGHAM, R.E. (1981) Capacity of 'transplanted' lymphocytes to traverse the intestinal epithelium of adult rats. *Transplantation*, **32,** 308.

SELBY, W.S., JANOSSY, G., BOFILL, M., GOLDSTEIN, G. & JEWELL, D.P. (1982) Mucosal lymphocyte subpopulations in inflammatory bowel disease. (abstr.) *Gastroenterology*, **23,** A896.

TAGLIABUE, A., LUINI, W., SOLDATESCHI, D. & BORASCHI, D. (1981) Natural killer activity of gut mucosal lymphoid cells in mice. *Eur. J. Immunol.* **11,** 919.

TAGLIABUE, A., BEFUS, A.D., CLARK, D.A. & BIENENSTOCK, J. (1982) Characteristics of natural killer cells in the murine intestinal epithelium and lamina propria. *J. exp. Med.* **155,** 1785.

THOMAS, J. & ANDERSON, N.W. (1982) Intraepithelial lymphocytes in the small intestinal mucosa of conventionally reared dogs. *Am. J. Vet. Res.* **43,** 200.

WEBB, M., MASON, D.W. & WILLIAMS, A.F. (1979) Inhibition of mixed lymphocyte responses by monoclonal antibody for a rat T lymphocyte subset. *Nature*, **282,** 841.