Virchows Archiv A Pathol Anat (1992) 420:419–424

*Virchows Archiv A*
*Pathological Anatomy and Histopathology*


# The distribution and possible function of gamma interferon-immunoreactive cells in normal endometrium and myometrium

**Colin J.R. Stewart, Maura A. Farquharson, and Alan K. Foulis**

Department of Pathology, The Royal Infirmary, Glasgow, UK

Received December 9, 1991 / Accepted January 14, 1992

**Summary.** T-lymphocytes are present in normal endometrium, where they may have a role in the control of glandular maturation. T-cell activity could be related to the local secretion of cytokines such as gamma interferon, which has an anti-proliferative effect on endometrial epithelial cells in vitro. We have examined gamma interferon immunoreactivity and T-cell distribution in 24 normal pre-menopausal uteri. Endometrial appearances were representative of all stages of the menstrual cycle. Most cells in the lymphoid aggregates in the stratum basalis were stained by T-cell and gamma interferon antisera. T-lymphocytes were also scattered in glandular epithelium and throughout the stroma of basal and functional layers; immunoreactivity for gamma interferon was less consistent in these cells. There was no alteration in the intensity or distribution of gamma interferon staining in different phases of the menstrual cycle. Endometrial granulocytes (K-cells) present mainly in the late secretory endometria were not reactive with the gamma interferon antiserum. In addition to endometrial staining, T-cells were distributed in all areas of the myometrium in most uteri, and many myometrial lymphocytes were gamma interferon positive. These results support a role for gamma interferon in endometrial physiology, possibly as an inhibitor of epithelial proliferation.

**Key words:** Endometrium – Myometrium – Lymphocytes – Gamma interferon

## Introduction

The presence of leucocytes in normal human endometrium is well documented. Studies by Kamat and Isaacson (1987) and Marshall and Jones (1988) indicate that cells expressing the common leucocyte antigen account for 10–15% of all stromal cells in both the proliferative and secretory phases of the menstrual cycle. The leucocytic population consists mainly of macrophages and T-lymphocytes, except in the late secretory phase, when endometrial granulocytes account for a substantial proportion of stromal cells.

The function of endometrial lymphoid tissue is uncertain, although comparison with other mucosal sites raises the possibility of a protective role in the response to exogenous antigens (Morris et al. 1985). However, the lymphoid cells in endometrium differ in organisation and phenotype from the characteristic mucosa-associated lymphoid tissues of respiratory and gastrointestinal tracts. An alternative role for endometrial lymphoid tissue has been proposed by Tabibzadeh and co-workers (1986a, 1988, 1989), who have suggested that lymphocytes may modulate endometrial glandular proliferation. The presence of distinct T-cell aggregates in the stratum basalis, where proliferative and secretory activity is least expressed, raises the possibility that lymphoid cells exert an inhibitory effect on epithelial maturation in this area. T-cell activity could be related to the local secretion of cytokines such as gamma interferon, which has been shown to have an anti-proliferative effect on endometrial epithelial cells in vitro (Tabibzadeh et al. 1988). To investigate further the possible role of endometrial T-cells in vivo, we have examined the distribution of immunoreactive gamma interferon-containing cells in surgically resected normal human uterus.

## Materials and methods

### *Tissue*

Formalin-fixed, paraffin-embedded blocks of normal pre-menopausal uterus were obtained from the pathology files of Glasgow Royal Infirmary. Cases selected were from patients undergoing hysterectomy for cervical disease (cervical intra-epithelial neoplasia or stage 1 cervical carcinoma) or benign ovarian pathology. Patients on current hormonal therapy or with a history of menstrual abnormality were excluded. The age range was 26–54 with a mean of 38.3 years.

A total of 24 uteri were examined. The endometrial appearances were representative of all stages of the menstrual cycle. Using stan-

*Offprint requests to:* A.K. Foulis

dard criteria (Noyes et al. 1950), endometria were allocated to early($n=5$), mid($n=4$) and late($n=3$) proliferative phases, and early($n=5$), mid($n=2$) and late($n=5$) secretory phases.

### *Immunocytochemistry*

Sections 4 μm thick were mounted on silane-coated slides. All cases were stained using a standard indirect immunoperoxidase technique and diaminobenzidine substrate with the following primary antisera: (1) mouse monoclonal antibody MT 1 (CD43, Clonab, Solihull, UK) 1/50 to stain T-cells (Ng et al. 1988); (2) mouse monoclonal antibody UCHL 1 (CD45RO, Dako, High Wycombe, UK) 1/100 to stain T-cells (Norton et al. 1986); (3) sheep polyclonal antiserum to gamma interferon (gift from K. Cantell, Helsinki). The specificity, working dilution and methodology for the interferon antiserum were as previously described (Hamilton et al. 1991). The following peroxidase conjugated secondary antisera were used: (1) rabbit anti-mouse immunoglobulin (Dako) 1/50; (2) swine anti-sheep immunoglobulin (Serotec, Oxford, UK) 1/100. Control sections were incubated with the appropriate normal mouse or sheep sera in place of primary antisera.

## Results

Immunocytochemical staining with the antisera UCHL 1 and MT 1 showed lymphoid cells to be distributed mainly in stratum basalis, where they formed distinct aggregates in relation to endometrial glands (Fig. 1). Lymphoid aggregates were less frequently observed in the deeper stratum functionalis (Fig. 2). A minority of lymphocytes within these aggregates was unstained by either antiserum. Smaller groups of immunoreactive cells were noted around basal vessels and single T-cells were also distributed apparently at random within the stroma of both basal and functional layers. Intra-epithelial lymphocytes were observed in most endometria, occasionally in relation to basal aggregates but also within glands of the functional layer (Fig. 3) and in surface epithelium. The antibodies MT 1 and UCHL 1 also stained neutrophil polymorphs.

In general, no changes were observed in the number or distribution of T-cells in different phases of the cycle. An exception, however, was the increase in UCHL 1 and MT 1 immunoreactive cells in the stratum functionalis in late secretory and shedding phases. Many of these cells had the morphological characteristics of endometrial granulocytes (K-cells). However, not all cells considered to be endometrial granulocytes appeared to stain with these T-cell antisera.

Although the distribution of lymphoid aggregates did not appear to alter throughout the menstrual cycle, the

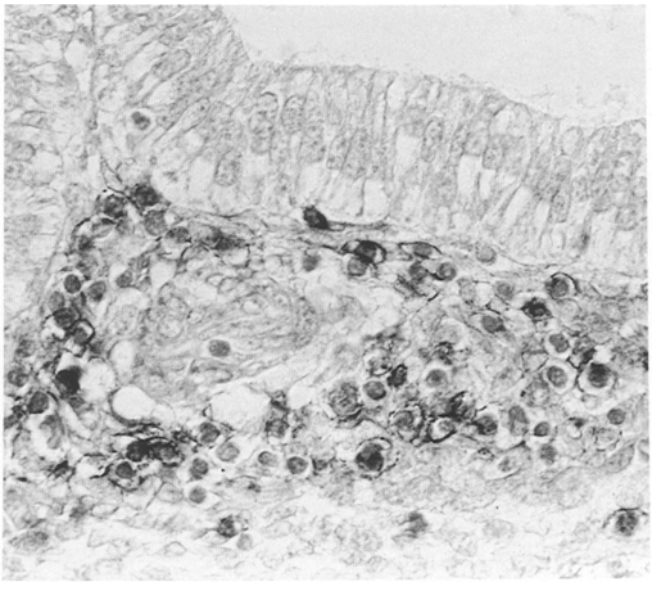

**Fig. 2.** Early secretory phase endometrium. Perivascular T-cell aggregate in stratum functionalis. UCHL 1, × 500

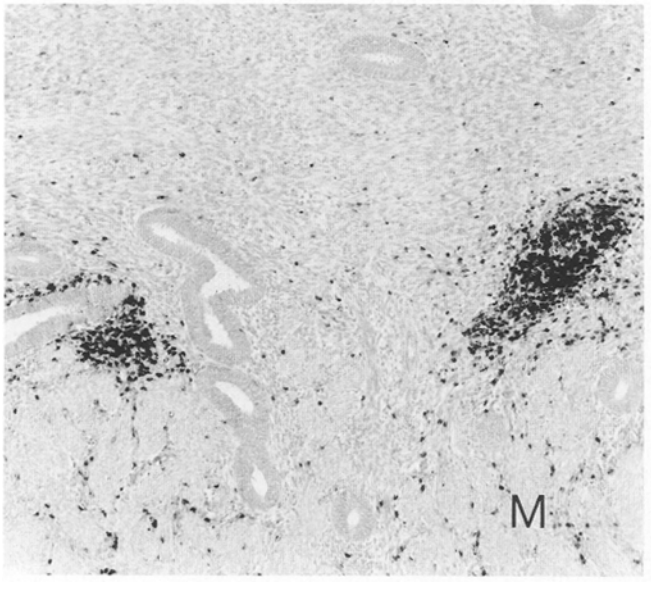


**Fig. 1.** Early proliferative phase endometrium. T-cells form distinct aggregates in stratum basalis. Single immunoreactive cells are also present in the superficial myometrium (*M*). UCHL 1, × 80

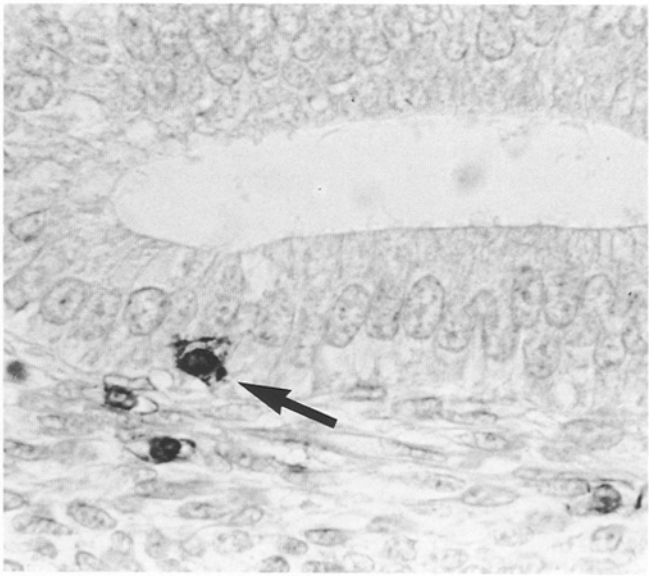

**Fig. 3.** Early secretory phase endometrium. Occasional lymphocytes are present within glandular epithelium (*arrow*). UCHL 1, × 785

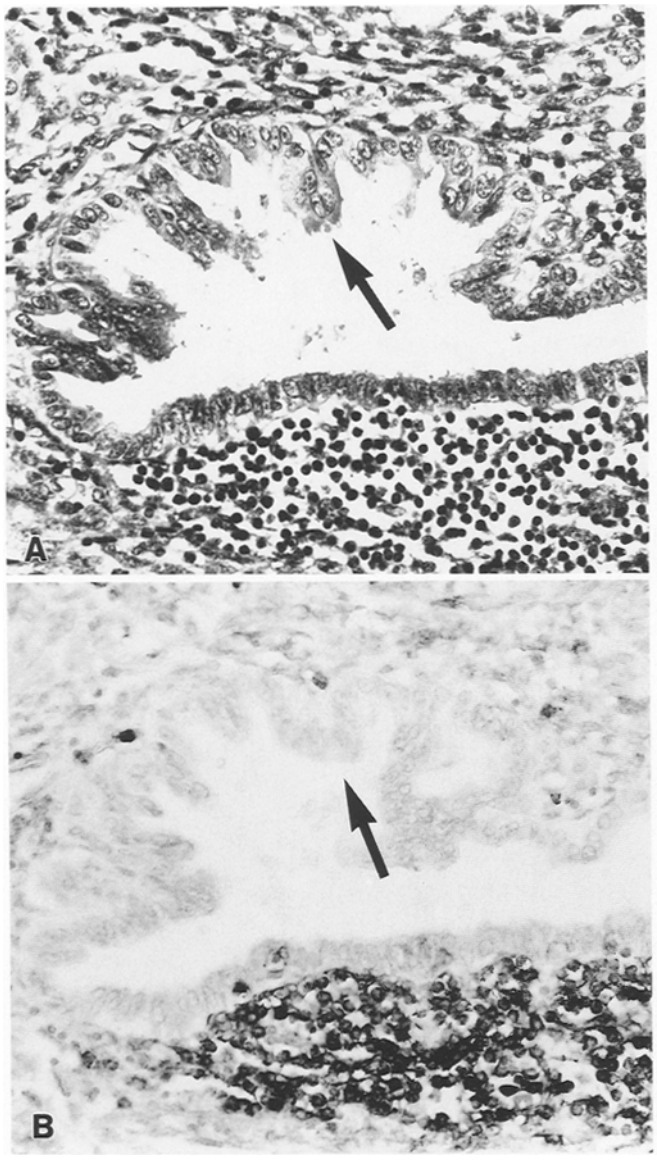


**Fig. 4A, B.** Late secretory phase endometrium. T-cell aggregate adjacent to immature basal-type epithelium. The superficial part of the gland shows secretory changes (*arrow*). **A** H & E; **B** UCHL 1, ×315

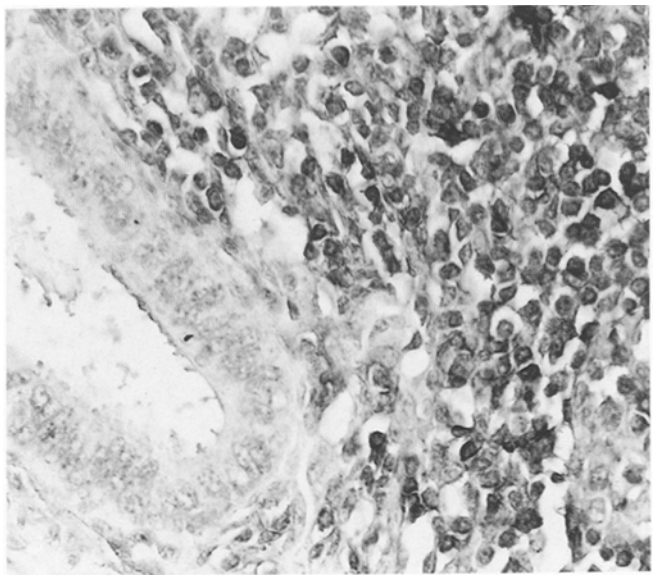

**Fig. 5.** Early proliferative phase endometrium. Lymphocytes in a basal aggregate are immunoreactive for gamma interferon. ×490

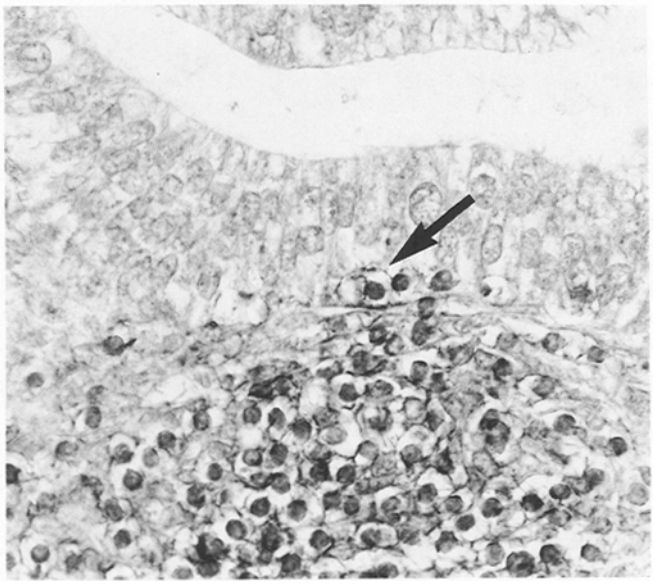

**Fig. 6.** Early secretory phase endometrium. Lymphoid aggregate in stratum functionalis and intraepithelial lymphocytes (*arrow*) are immunoreactive for gamma interferon. ×565

epithelium associated with T-cell areas tended to show the least proliferative or secretory development. In a few cases, the demarcation between basal-type and secretory epithelium in a single gland appeared to be closely related to the presence of stromal lymphoid elements (Fig. 4A, B).

Gamma interferon immunoreactivity was constantly observed in the lymphoid cells in basal aggregates (Fig. 5). The intensity of staining varied between cases but without any clear relationship to phases in the menstrual cycle. The perivascular groups in basalis and the occasional lymphoid aggregates in the deeper functional layer were also stained. Immunoreactivity for gamma interferon was less constant in the scattered lymphocytes in the functionalis and in general fewer cells were stained than with T-cell antisera. The random distribution of immunoreactive cells was, however, maintained and again there was no clear relationship with endometrial cyclical changes. Intra-epithelial lymphocytes similarly gave an inconsistent pattern of staining with the interferon antiserum; immunoreactivity was focally present (Fig. 6), but most cells were not stained, and in some instances there was a discrepancy between adjacent stromal and intra-epithelial lymphocytes. Endometrial granulocytes in the late secretory and shedding endometria were not stained (Fig. 7A, B). The gamma interferon antiserum also produced inconsistent staining of glandular epithelium, particularly apical cytoplasm; as non-immune sheep serum gave a similar pattern, this staining was discounted.

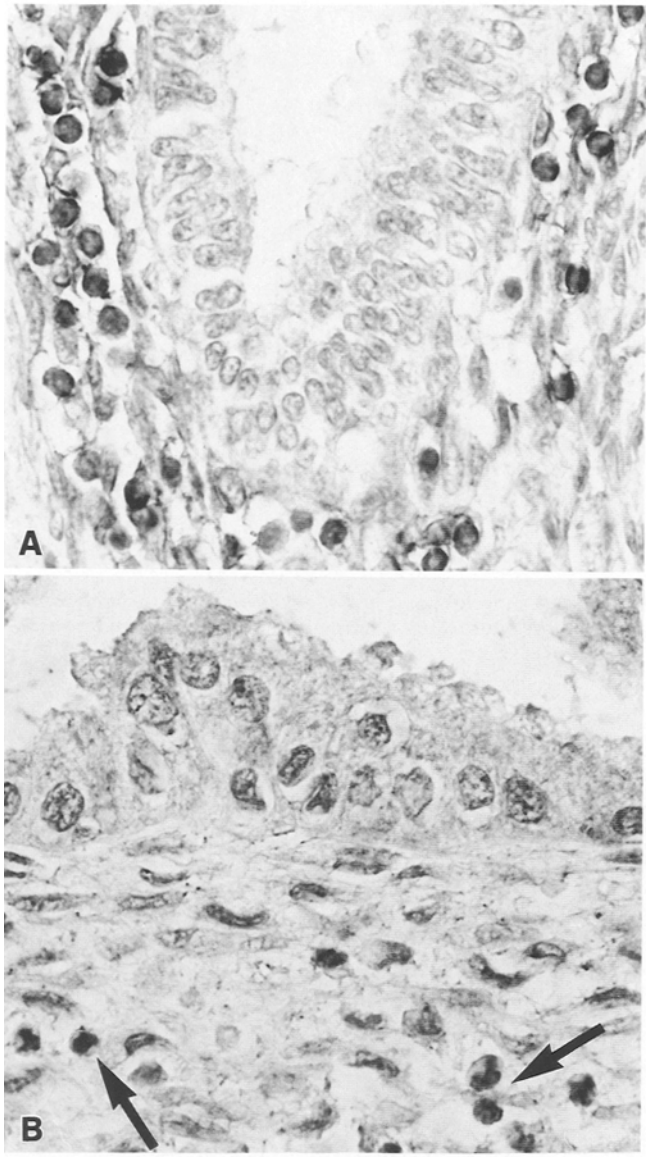


**Fig. 7.** Late secretory phase endometrium. Gamma interferon antiserum stains lymphocytes in basal endometrium (**A**) but not endometrial granulocytes (*arrows*) in functionalis (**B**) in the same biopsy. × 785

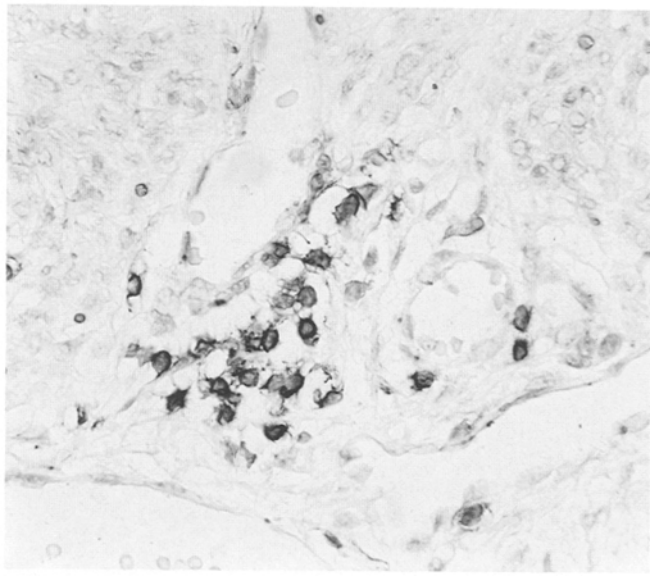

**Fig. 8.** Myometrium; there are small T-cell aggregates around myometrial vessels. UCHL 1, × 500

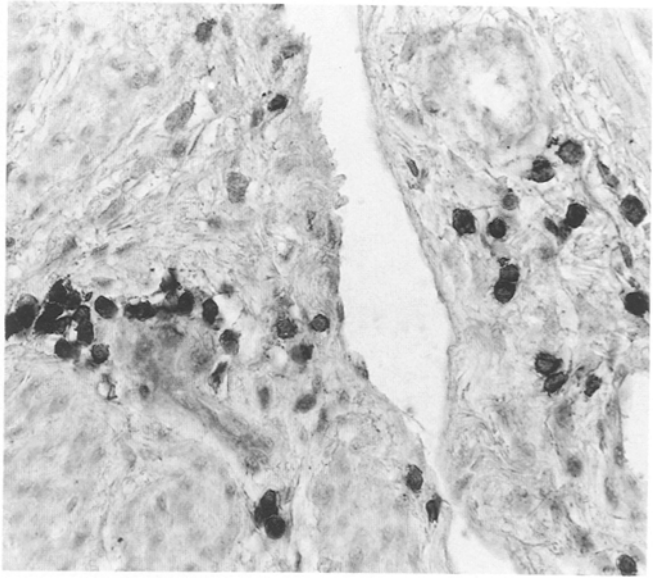

**Fig. 9.** Myometrium; lymphocytes are immunoreactive for gamma interferon. × 390

While the major purpose of this study was to examine endometrium, it was also clear that surprisingly frequent lymphocytes were present in the myometrium in most uteri (Fig. 8). These were distributed singly and in small groups of up to 5–10 cells particularly around small vessels. Examination of corresponding H & E-stained sections suggested that the latter were related to small foci of spindle cells which morphologically resembled endometrial stromal cells. None of the uteri examined showed evidence of adenomyosis. Lymphoid cells appeared more prominent in the muscle adjacent to the endometrial junction but were distributed in all areas of the myometrium. Like the cells in the stratum basalis, most myometrial lymphocytes were stained with T-cell antisera and were immunoreactive with gamma interferon (Fig. 9).

## Discussion

In this study we have shown that T-lymphocytes are present in significant numbers in normal endometrium, where they form distinct lymphoid aggregates, most commonly in the stratum basalis. Single cells are also distributed throughout the endometrial stroma and within glandular and surface epithelium. These findings are in keeping with previous immunocytochemical studies of endometrium, which have also demonstrated the predominance of CD8-positive T-cells in all areas (Kamat and Isaacson 1987; Marshall and Jones 1988; Morris et al. 1985).

The function of lymphoid tissue in endometrium is uncertain, but a protective role in the recognition and response to exogenous antigens has been suggested. Endometrial lymphoid cells could therefore be regarded as forming a mucosa-associated lymphoid tissue (Morris et al. 1985). However, the endometrium differs in several important aspects from the mucosae of gastrointestinal and respiratory tracts. The endometrial milieu is normally sterile, and physiological immunogenic challenges are restricted to those associated with implantation and pregnancy. Menstruation leads to cyclical loss of the functionalis including its associated lymphoid component. Furthermore, endometrial mucosa is unique in that most lymphocytes are phenotypically T-cells, while B-cell elements including germinal centres and plasma cells are scarce or absent.

An alternative role for lymphoid tissue in endometrial physiology has been proposed by Tabibzadeh and coworkers (1986a, 1988, 1989), who have suggested that lymphocytes, like other stromal cells, may have a modulatory role in glandular proliferation. It is accepted that oestrogenic and progestogenic hormones are the major influences in endometrial maturation, but the difference in response between basal and functional layers is not explained. The close association of T-lymphoid aggregates with basal epithelium would be consistent with the release of T-cell-derived cytokines with a local inhibitory effect on glandular proliferation. Gamma interferon, which is secreted by activated T-cells (Klein et al. 1982), inhibits endometrial epithelial proliferation in vitro (Tabibzadeh et al. 1988), and gamma interferon receptor can be detected on glandular cells of normal endometrium using immunocytochemical techniques (Tabibzadeh 1990). Indirect support for gamma interferon release in the stratum basalis is also provided by the selective distribution of class II major histocompatability complex (MHC) antigens on epithelium in this area (Morris et al. 1985; Tabibzadeh et al. 1986b). Gamma interferon induces class II MHC expression on many types of cell including endometrial epithelial cells in vitro (Tabibzadeh et al. 1986a). In this study, the immunolocalisation of gamma interferon in T-cell areas is further evidence for a role in endometrial physiology. Local release of T-cell gamma interferon could be under oestrogenic control, as oestrogen receptors have been detected immunocytochemically in endometrial lymphoid cells (Tabibzadeh and Satyaswaroop 1989). No clear alteration in cell distribution or interferon immunoreactivity was observed in different phases of the menstrual cycle and it is likely that other factors, possibly derived from lymphocytes or other stromal cells, are also involved in the control of epithelial development in the endometrium.

The function of intra-epithelial lymphocytes and scattered T-cells within the functional layer is uncertain. Both groups of cells were less consistently immunoreactive for gamma interferon than those in lymphoid aggregates. Intra-epithelial lymphocytes in gastrointestinal mucosa, like those in endometrium, are predominantly CD8-positive T-cells and have been shown to produce gamma interferon in response to phytohaemagglutinin in vitro (Ebert 1990). Gut intra-epithelial lymphocytes differ from peripheral blood cells in mitogenic responses and may have a specialised role in the reaction to intestinal antigens; the functional relationship to these cells to their endometrial counterparts is, however, not known.

Endometrial granulocytes were prominent in late secretory endometria and many of these cells were immunoreactive for UCHL 1 and MT 1. This is in keeping with previous immunocytochemical studies particularly in decidualised endometrium (Bulmer et al. 1987; Pace et al. 1989). Endometrial granulocytes are considered to represent a specialised lymphoid subset sharing some T-cell and natural killer cell surface antigens, and may have an immunomodulatory role in embryo implantation (Ritson and Bulmer 1987). It is interesting that endometrial granulocytes were not immunoreactive for gamma interferon; experimentally, gamma interferon has adverse effects on early embryo growth and trophoblast development (Johnson 1989).

The presence of lymphocytes in normal myometrium has not been previously documented in detail, although Ismail (1990) commented on scanty collections of mature myometrial lymphocytes in hysterectomy specimens. The T-cell antisera revealed surprisingly numerous single cells and small aggregates throughout the myometrium in most uteri examined in this study. Many cells were immunoreactive for gamma interferon. The function of these lymphocytes is uncertain, but a physiological role similar to that of cells in endometrium is at least a possibility. It is of interest that in the rare uterine leiomyomas with marked lymphoid infiltration, many of the reactive cells were UCHL 1 positive (Ferry et al. 1989).

## References


Bulmer JN, Hollings D, Ritson A (1987) Immunocytochemical evidence that endometrial stromal granulocytes are granulated lymphocytes. J Pathol 153:281–288

Ebert EC (1990) Intra-epithelial lymphocytes: interferon-gamma production and suppressor/cytotoxic activities. Clin Exp Immunol 82:81–85

Ferry JA, Harris NL, Scully RE (1989) Uterine leiomyomas with lymphoid infiltration simulating lymphoma. Int J Gynecol Pathol 8:263–270

Hamilton F, Black M, Farquharson MA, et al (1991) Spatial correlation between thyroid epithelial cells expressing class II MHC molecules and interferon-gamma-containing lymphocytes in human thyroid autoimmune disease. Clin Exp Immunol 83:64–68

Ismail SM (1990) Follicular myometritis, a previously undescribed component of pelvic inflammatory disease. Histopathology 16:91–93

Johnson PM (1989) Immunological intercourse at the feto-maternal interface. Immunol Today 10:215–217

Kamat BR, Isaacson PG (1987) The immunocytochemical distribution of leukocytic subpopulations in human endometrium. Am J Pathol 127:66–73

Klein JR, Raulet DH, Pasternack MS, Bevan MJ (1982) Cytotoxic T lymphocytes produce immune interferon in response to antigen or mitogen. J Exp Med 155:1198–1203

Marshall RJ, Jones DB (1988) An immunohistochemical study of lymphoid tissue in human endometrium. Int J Gynecol Pathol 7:225–235

Morris H, Edwards J, Tiltman A, Emms M (1985) Endometrial lymphoid tissue: an immunohistological study. J Clin Pathol 38:644–652

Ng CS, Chan JKC, Hui PK, Lo STH (1988) Monoclonal antibodies reactive with normal and neoplastic T cells in paraffin sections. Hum Pathol 19:295–303

Norton AJ, Ramsay AD, Smith SH, et al (1986) Monoclonal antibody (UCHL 1) that recognises normal and neoplastic T cells in routinely fixed tissues. J Clin Pathol 39:399–405

Noyes RW, Hertig AT, Rock J (1950) Dating the endometrial biopsy. Fertil Steril 1:3–25

Pace D, Morrison L, Bulmer JN (1989) Proliferative activity in endometrial stromal granulocytes throughout menstrual cycle and early pregnancy. J Clin Pathol 42:35–39

Ritson A, Bulmer JN (1987) Endometrial granulocytes in human decidua react with a natural-killer (NK) cell marker, NKH 1. Immunology 62:329–331

Tabibzadeh S (1990) evidence of T-cell activation and potential cytokine action in human endometrium. J Clin Endocrinol Metab 71:645–649

Tabibzadeh SS, Satyaswaroop PG (1989) Sex steroid receptors in lymphoid cells of human endometrium. Am J Clin Pathol 91:656–663

Tabibzadeh SS, Gerber MA, Satyaswaroop PG (1986a) Induction of HLA-DR antigen expression in human endometrial epithelial cells in vitro by recombinant gamma-interferon. Am J Pathol 125:90–96

Tabibzadeh SS, Bettica A, Gerber MA (1986b) Variable expression of Ia antigens in human endometrium and in chronic endometritis. Am J Clin Pathol 86:153–160

Tabibzadeh SS, Satyaswaroop PG, Rao PN (1988) Anti-proliferative effect of interferon-gamma in human endometrial epithelial cells in vitro: potential local growth modulatory role in endometrium. J Clin Endocrinol Metab 67:131–138