Immunology 1992 77 515-519

The distribution of immunoreactive interferon-γ-containing cells in normal human tissues

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Accepted for publication 8 July 1992

SUMMARY

An immunohistochemical technique has been used to study the distribution of lymphocytes expressing interferon-γ in normal adult tissues. The greatest concentrations of these cells were seen in mucosal sites exposed to a resident microflora. It is proposed that such organisms, by eliciting immune responses, provide the stimulus for the production of 'physiological' interferon-γ. This in turn may act to preserve the 'tone' or readiness of the immune system.

INTRODUCTION

The concept of 'physiological' interferon—that is the continual production of interferons in health—was first promulgated by Bocci.[1,2] He argued that since an animal with an immune system is under constant antigenic challenge from the resident microbial flora and from food antigens, the immune system must be constantly stimulated, with subsequent production and release of cytokines by cells of the immune system.

We recently addressed part of this problem by examining foetal, newborn and adult human tissues for the presence of immunoreactive interferon-α.[3,4] Interferon-α was present in a proportion of macrophages in tissues from adults, newborns and foetuses older than 9 weeks gestation. There was an increased frequency of interferon-α-containing macrophages in bronchi of newborn infants compared with bronchi of foetuses, suggesting induction of interferon-α upon exposure to a microbial flora after birth.[4] An unexpected finding was that interferon-α was also present in parenchymal cells of the choroid plexus in brain, pituitary, thyroid, adrenal and parathyroid in all age groups.

The development of an immunocytochemical technique capable of detecting interferon-γ in paraffin-embedded formalin-fixed tissues[5] now permits similar investigations on the distribution of interferon-γ positive cells to be done. The current study aims to define which cells in normal human adult tissues express interferon-γ and assesses whether the distribution of these cells is consistent with Bocci's hypothesis that the microbial flora is a major stimulus to physiological interferon production.

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MATERIALS AND METHODS

Tissues studied

The surgical pathology and autopsy files of the Royal Infirmary, Glasgow, were searched to identify formol saline-fixed paraffin-embedded blocks of normal human tissues. Normal tissues were commonly taken from excision margins of tumour resections, but histologically normal biopsies, and tissues removed inadvertently (e.g. thymus during parathyroidectomy), or for surgical access (e.g. spleen during distal pancreatectomy) were also included. As immunoreactive interferon-γ appears to survive well in post-mortem tissues,[6] autopsy samples of normal thyroid, heart, adrenal and brain were included. None of the patients from whom these samples were taken had clinical evidence of sepsis or viral infection.

The tissues studied included tonsil (10), tongue (2), salivary gland (3), stomach (4), duodenum (5), jejunum (7), appendix (11), colon (10), liver (3), gallbladder (4), pancreas (4), renal cortex (3), renal pelvis (3), ureter (3), urinary bladder (4), prostate (8), testes (5), ovary (8), fallopian tube (16), endometrium (9), cervix (9), female breast (10), skeletal muscle (3), myocardium (4), adrenal (9), thyroid (10), cerebral cortex (3), choroid plexus (3), main bronchus (4), lung, including intrapulmonary bronchi (3), skin (5), adult thymus (8), unreactive lymph nodes from various sites (cervical (7), axillary (7), mesenteric (5), inguinal (2), bone marrow (10), spleen (9) and peripheral blood leucocytes (4). The peripheral blood leucocytes were taken from the buffy coats of normal volunteers, fixed in formol saline and embedded in paraffin wax.

Immunocytochemical detection of interferon-γ

Four micron-thick serial sections were cut from the paraffin-embedded tissues and mounted on poly-L-lysine-coated slides. Section 1 was stained by haematoxylin and eosin. Section 2 was stained by an indirect immunoperoxidase technique for inter-

feron-γ using as the primary antiserum a sheep polyclonal antiserum to highly purified human interferon-γ (gift from K. Cantell, National Public Health Institute, Helsinki, Finland). In section 3, the sheep antiserum was substituted by normal sheep serum (SAPU, Law, Scotland) to act as a negative control. Diluted antiserum and normal sheep serum used in the control studies were absorbed with a mixture of guinea-pig and porcine liver powders (Sigma, Poole, U.K.) before their use in the indirect immunoperoxidase technique. This procedure reduced non-specific binding to fixed human tissues. The antiserum to interferon-γ was used at a final dilution of 1:100. The secondary antiserum, peroxidase-conjugated swine anti-sheep immunoglobulins (Serotec, Oxford, U.K.), was used at a dilution of 1/100.

Details of the specificity of the interferon-γ antiserum have been given previously.[5,7] The antiserum showed no detectable activity against human interferon-α or interferon-β.[7] In a variety of chronic inflammatory conditions in humans including chronic pancreatitis, type 1 diabetes and autoimmune thyroid disease, we have shown that the indirect immunoperoxidase technique described above stained small lymphocytes.[5,6] This staining was not present if the antiserum was absorbed with recombinant human interferon-γ (Genentech, South San Francisco, CA) prior to use.[5]

In studies of gut tissues, additional sections were stained by indirect immunoperoxidase techniques with the mouse monoclonal primary antibodies, L26 (CD20) (Dako, High Wycombe, U.K.), a B-lymphocyte marker, and α-T (CD43) (Alpha Laboratories, Eastleigh, U.K.) which identifies T lymphocytes. Peroxidase-conjugated rabbit anti-mouse immunoglobulin (Dako) was used as secondary antiserum. Diaminobenzidine was used as the substrate in all the reactions.

Assessment of results

The primary purpose of this study was to describe the anatomical distribution of interferon-γ-containing cells in normal tissues. In addition, a quantitative assessment of the number of small lymphocytes expressing interferon-γ in each organ was attempted. An arbitrary score of ++++ was given for the tissue with the highest density of positive lymphocytes (jejunal mucosa) and the staining in other organs was then scored by reference to this baseline. A more accurate scoring system was precluded by the non-random distribution of lymphocytes within individual tissues (*vide infra*).

RESULTS

General distribution of interferon-γ-containing cells

Immunocytochemistry for interferon-γ stained cells with the morphology of small lymphocytes in peripheral blood and in interstitial tissues of the majority of organs (Table 1). No parenchymal cells were positive in any tissue and there was no convincing evidence that other leucocytes, accessory lymphoid cells, immunoblasts, macrophages or mast cells contained this cytokine. Positively staining lymphocytes showed a characteristic appearance with a rim of positivity around the nucleus (Fig. 1).

It can be seen from Table 1 that skin and tissues which constitute the mucosal-associated lymphoid system were most likely to contain positive cells. By contrast, tissues such as brain, skeletal muscle, renal cortex and medulla, myocardium, non-lactating breast, and endocrine organs contained negligible numbers of interferon-γ positive lymphocytes.

Table 1. Relative distribution of interferon-γ-containing lymphocytes in various organs

Gastrointestinal tract mucosae and associated organs		
Tongue +++	Salivary gland ++	Stomach +++
Duodenum ++++	Jejunum ++++	Appendix ++
Colon +++	Liver ++	Gallbladder +
Pancreas ±		
Respiratory tract		
Bronchi +++	Alveoli ++	
Genitourinary organs		
Ovary ++	Fallopian tube +	Endometrium +++
Cervix +++	Prostate +++	Testes −
Renal cortex ±	Urothelium +	
Endocrine organs		
Thyroid ±	Adrenal +	
Lymphoid organs		
Lymph nodes ++	Tonsil ++	Thymus ++
Spleen ±	Bone marrow +	Peripheral blood ++++
Others		
Skin ++	Breast +	Skeletal muscle −
Cardiac muscle ±	Cerebral cortex −	Choroid plexus −

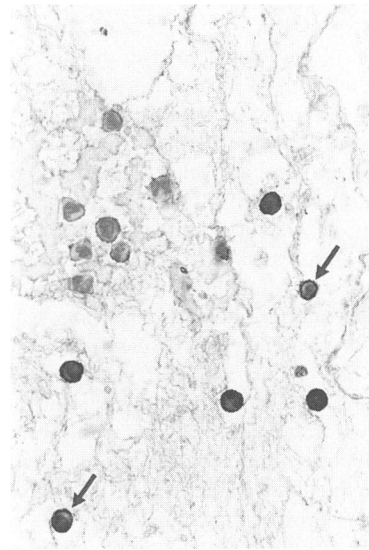

Figure 1. Interferon-γ in peripheral blood leucocytes embedded in a fibrin clot. Several densely stained lymphocytes are present. The peripheral rim of cytoplasmic staining is clearly visible in two cells (arrows). (Indirect immunoperoxidase (II) for interferon-gamma: original magnification ×800.)

Gastrointestinal tract

Lamina propria lymphocytes containing interferon-γ were found throughout the gastrointestinal tract, with the greatest

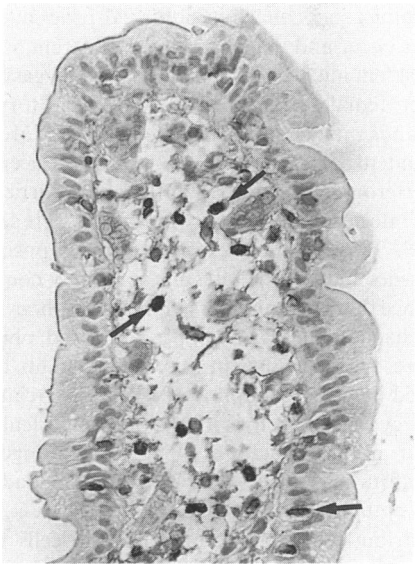

Figure 2. Small intestinal villus stained for interferon-γ. Examples of positively stained lymphocytes are arrowed (original magnification ×490).

frequency being seen in the small bowel (Fig. 2). Up to 50% of lamina propria lymphocytes in jejunum were interferon-γ positive, but only about 10% of intraepithelial lymphocytes expressed this product.

The gut-associated lymphoid tissues, such as tonsil, Peyer's patches and lymphoepithelial complexes of the appendix and colon, showed a fairly uniform pattern of staining. On analysis of adjacent sections stained to show B and T lymphocytes respectively, it was seen that interferon-γ positive cells were absent from B-cell areas (e.g. germinal centres). Lymphocytes lying in the zone between the germinal centre and the lumen of the gut (dome area) were usually negative, as were cells in the vicinity of high endothelial venules. However, large numbers of positive lymphocytes were found at the deep periphery of the T zone (Fig. 3). Draining lymphatics deep to tonsil or in the mesoappendix contained many positive lymphocytes.

Genitourinary tract

Lymphoid aggregates were present particularly in the lower (non-proliferating) third of the endometrium. Many cells in these aggregates were positive, as were submucosal lymphocytes at the junction between endocervix and ectocervix. However endometrial granulocytes (K cells) were negative. In the ovary, most positive cells were found in degenerating corpora lutea. Many positive lymphocytes were present in interstitial tissue in prostate.

Lymphoid system

Lymph nodes were sampled from a wide variety of sites but the findings were essentially similar throughout. Positive cells were found within afferent peripheral sinuses and medullary sinuses (Figs 4 and 5). Lymphocytes in B-cell zones were negative and only occasional positive cells were seen in the T-cell-dependent zones. Cells traversing high endothelial venules were also negative. Scattered positive cells were present at the periphery of thymic cortex but few positive cells were seen in the spleen.

Other tissues

Submucosal lymphocytes are common in bronchus and some lymphoid aggregates are present among lung alveoli. The bronchial lymphocytes were particularly likely to be positive for interferon-γ. Interferon-γ positive lymphocytes in the skin were predominantly perivascular and were found in the superficial dermis.

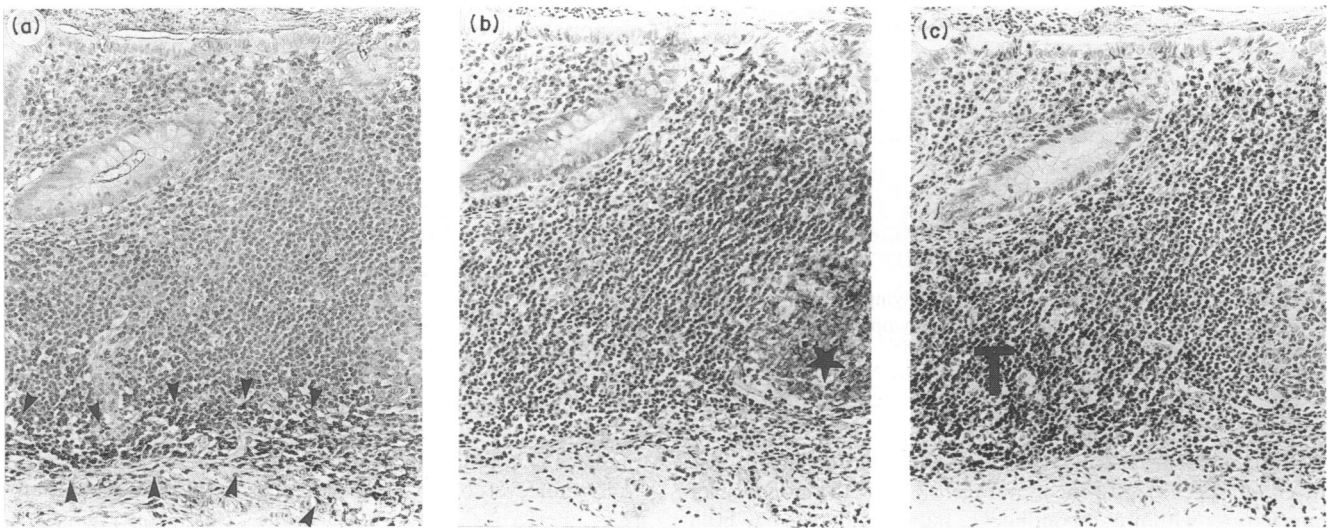

Figure 3. Normal large bowel mucosa (appendix). The lumen is at the top of the photograph and the muscularis mucosae at the bottom. This lymphoepithelial complex has been stained to show interferon-γ positive cells (a), B lymphocytes (CD20) (b) and T lymphocytes (CD43) (c). Note that B lymphocytes are aggregated around the germinal centre (asterisk) and that the T lymphocytes are aggregated below and to the left of this (T). Interferon-γ positive cells lie along the base of the lymphoepithelial complex adjacent to the muscularis mucosae (area between arrow heads) (original magnification ×200).

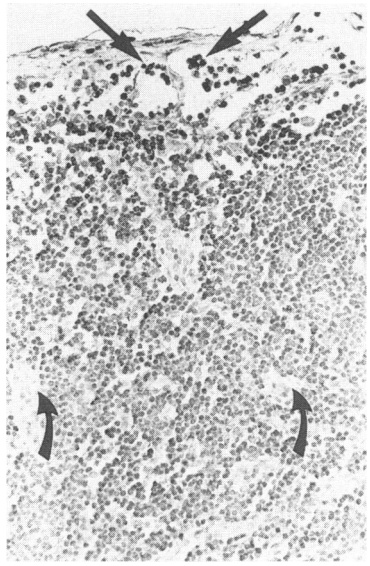

Figure 4. Periphery of normal lymph node. Darkly staining aggregates of interferon-γ positive lymphocytes (straight arrows) are present in the peripheral sinus. Such cells are not seen adjacent to high endothelial venules (curved arrows). (II for interferon-γ: original magnification × 320.)

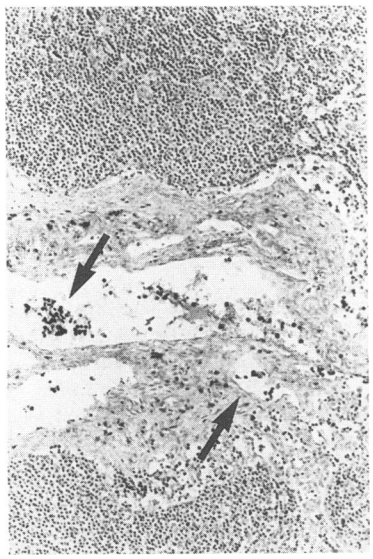

Figure 5. Aggregates of interferon-γ positive lymphocytes are largely confined to medullary sinuses of this normal lymph node (arrows). (II for interferon-γ: original magnification × 125.)

DISCUSSION

T lymphocytes are stimulated to secrete interferon-γ in response to either specific antigen or mitogens;[8] thus the presence of interferon-γ within a T lymphocyte indicates that it has been activated. The present study was an attempt to study the degree of T-cell activation in physiological conditions in humans by examining tissues for the presence of interferon-γ, thereby addressing the problem of whether there was or was not production of 'physiological' interferon.

Our findings show that physiological production of interferon-γ does occur and that this is most apparent at sites where there is a resident microflora, including the skin, gastrointestinal tract, lower female genital tract and respiratory passages. Salivary glands may be included here since they are frequently the site of latent viral infection.[9] A second inducer of physiological interferon-γ may be food antigens, as particularly large numbers of interferon-γ positive lymphocytes were seen in upper small bowel. The prominence of interferon-γ positive cells in mucosal tissues such as the intestine is entirely consistent with other evidence that large numbers of the T lymphocytes found in these sites have an activated appearance and phenotype.[10,11] Furthermore, recent studies in mice and humans have shown that isolated mucosal lymphocytes produce relatively large amounts of cytokines such as interferon-γ, interleukin-5 (IL-5) and tumour necrosis factor-α (TNF-α).[12-14] Together, these findings confirm that the physiological antigen load is a potent activation stimulus to mucosal lymphoid cells.

The distribution of interferon-γ positive cells within lymphoid organs may also give some idea of the circulation pathway which they take. The presence of such cells in peripheral and medullary sinuses of lymph nodes, their absence in the vicinity of high endothelial venules and their limited numbers in T-cell-dependent areas within nodes suggest that such cells are primarily passing through nodes and are not derived from cells which enter the node from the bloodstream. These interferon-γ positive cells may therefore represent lymphocytes which have emigrated from peripheral blood directly into tissues such as dermis, or the lamina propria of mucosae, before draining into afferent lymph. It is not clear from this study whether such emigrating cells are derived from lymphocytes in peripheral blood which are already interferon-γ positive, or whether cells not expressing this cytokine are induced to do so on exposure to antigen in the tissue. A second possible source of interferon-γ positive lymphocytes in efferent lymph from lymph nodes may be lymphocytes which have been activated within the lymph node itself.

This circulation pathway is similar to that proposed for memory T cells in sheep, where it has been shown that such cells traffic from blood into peripheral tissues and thence into lymph nodes in afferent lymph, finally to return to the bloodstream via efferent lymphatics and the thoracic duct. In contrast, naive T lymphocytes enter lymph nodes from the bloodstream via high endothelial venules.[15] The fact that memory T cells have an enhanced ability to produce interferon-γ upon antigenic re-stimulation[16] is further evidence that many of the interferon-γ positive cells described in the present study may be memory T cells. It would be of interest to establish whether or not interferon-γ positive cells in normal tissues co-express markers of memory T cells, such as CD45RO.

It is important to emphasize that all the tissues used in this study, including the lymphoid organs, were normal and had no evidence of pathological processes. Thus, our findings reflect the presence of 'physiological' interferon-γ, presumably in response to normal levels of environmental antigen. In view of the many roles interferon-γ has in the immune system, this could have several consequences for the individual. Firstly, as has been hypothesized previously, the physiological production of cytokines such as interferon-γ may be required to maintain the 'tone' of the immune system in a state of constant readiness to defend organs such as mucosal tissues which are exposed to continual

antigenic challenge.[2] This hypothesis is supported by the finding that treatment of dogs with the immunosuppressive agent, cyclosporin A, an inhibitor of interferon-γ secretion, abolishes expression of class II major histocompatibility complex (MHC) by vascular endothelium.[17] Furthermore, germ-free animals have atrophic lymphoid organs,[18] make poor cell-mediated immune responses to novel antigens[19] and have low levels of macrophage and natural killer cell activity.[20,21] It would be important to confirm the potential role of interferon-γ in these latter phenomena by examining expression of interferon-γ in the tissues of foetuses, unweaned neonates and germ-free animals.

A second role of physiological interferon-γ may be to influence the structure and function of tissues with a large component of immune cells such as the intestinal mucosa. Germ-free animals or animals fed a low-antigen diet have a relatively immature intestinal epithelium which has a low cell turnover, poor differentiation and little or no expression of class II MHC molecules.[11,22] Normal intestinal anatomy is restored by increasing the antigen load. In addition, interferon-γ has several direct effects on intestinal epithelial cells *in vitro*, including increased expression of secretory component and class II MHC antigens, as well as modulation of cell proliferation and ion transport.[23-26] Thus the role of physiological interferon-γ in the growth and differentiation of normal intestine and other mucosal tissues deserves further study.

ACKNOWLEDGMENTS

Professor K. Cantell of the National Public Health Institute, Helsinki, Finland, kindly supplied the antiserum to interferon-gamma. A.M. is supported by the Coeliac Trust. Mrs J. Cramb kindly typed the manuscript.

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