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THE INFLUENCE OF LOCAL INFECTION ON IMMUNOGLOBULIN FORMATION IN THE HUMAN ENDOCERVIX

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SUMMARY

The formation of immunoglobulins in the lamina propria of the endocervix was studied in relation to specific acute local infection. Plasma cells containing IgA, IgG and IgM were identified immunohistochemically by the direct fluorescent antibody method in specimens obtained by needle biopsy. Infection by *Neisseria gonorrhoeae*, *Trichomonas vaginalis* or *Candida albicans* was associated with an increase in the numbers of fluorescing plasma cells in all three classes, but predominantly IgA; plasma cells of the IgM class were more prominent in trichomoniasis than in the other two infections. Sexual contacts of patients with gonorrhoea, in whom the organism could not be demonstrated, showed an identical response to bacteriologically confirmed cases, but contacts of patients with non-specific urethritis were indistinguishable from normals.

INTRODUCTION

The human endocervix is lined with secretory columnar epithelium which produces mucus shown to contain immunoglobulins (Moghissi & Neuhaus, 1962; Schumacher, Strauss & Wied, 1965; Hervé, Robey & Sergent, 1965; Elstein & Pollard, 1968). Specific antibodies in cervical mucus have been demonstrated against spermatozoa (Solish, Gershowitz & Behrman, 1961; Straus, 1965), blood group antigens, *Escherichia coli* and *C. albicans* (Parish, Carron-Brown & Richards, 1967). Local formation of antibody is suggested by the finding of higher titres in mucus than in serum (Parish *et al.*, 1967), the detection of secretory piece and lactoferrin in endocervical epithelium by immunofluorescence (Masson, Heremans & Ferin, 1968; Hulka & Omran, 1969; Tourville *et al.*, 1970), and the location of IgA- and IgG-containing plasma cells in the underlying stroma by the same method (Masson & Ferin, 1969). In the female reproductive tract local antibody production was first investigated in cows by Pierce (1947) who showed that vaginal secretions contained a higher titre of antibodies to *Trichomonas foetus* than serum, and this has subsequently been confirmed with experimentally introduced antigens in the rabbit (Batty & Warrack, 1955) and human (Straus, 1961). More recently, Bell and Wolf (1967) have demonstrated *in vitro*

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antibody synthesis by rabbit vaginal tissue following application of diphtheria toxoid *in vivo*, but no work has been reported on immunoglobulin production in response to acute infection of the human lower genital tract. In the present investigation we report the influence of local genital infection on the population of plasma cells in the submucosa of the endocervix using the direct fluorescent antibody technique on specimens obtained by needle biopsy.

PATIENTS AND METHODS

All the patients examined were attending a clinic for sexually transmitted diseases either as contacts of infected men, or because they had genital symptoms, or because they thought it desirable to be checked. They were almost entirely single women between 18 and 30 years of age; approximately 55% used oral contraceptives; 20% were parous and 30% had had miscarriages or terminations.

Material was obtained by suction-punch biopsy using an instrument adapted from the Abrams punch biopsy needle (Abrams, 1958). This was modified in four ways: the shaft was lengthened to 12 cm, the biopsy aperture was cut square, the end of the needle was rounded, and a stop was fitted to ensure a consistent biopsy site 0·5 cm from the external os. The specimen was a piece of tissue approximately 2 mm × 2 mm × 4 mm with epithelium lining a larger surface. The procedure was completely painless and produced slight but transitory bleeding; no complications have occurred in over 100 biopsies.

Tissue was either quick frozen immediately, or fixed in 10% neutral isotonic buffered formaldehyde and kept at 4°C, then washed overnight in 30% sucrose and quick frozen, according to the method of Eidelman & Davis (1968). Serial sections (5 μm thick) were prepared in a cryostat and fixed in a stream of cool air for 30 min.

The total plasma cell count and polymorphonuclear leucocyte infiltration were estimated on sections stained with haematoxylin and eosin. Tissue localization of IgA, IgG and IgM was assessed by staining with specific FITC-conjugated anti-human sera (Wellcome Reagents Ltd., Beckenham, Kent) and by examining at magnifications of 128 and 480 with a Reichert Zetopan fluorescence microscope using a Filtra-flex FITC 4 excitation filter (Balzers High Vacuum Ltd., Berkhamsted, Herts.) and a Kodak Wratten No. 12 gelatin barrier filter. Contrast was enhanced and autofluorescence eliminated by counterstaining with 0·02% Evans' blue, which produced a red background.

Examination of the sections was carried out by one of us (E.J.C.) without knowledge of the diagnosis of the patient from whom the specimen was taken. Plasma cell concentration was assessed on a three point scale (+, ++, +++); those sections which contained only a few fluorescing cells were designated $\pm$.

RESULTS

Patients included in the investigation fell into four basic diagnostic categories: cervical gonorrhoea, vaginal trichomoniasis, vaginal candidosis and no abnormality detected. The last group included some patients who attended as contacts of gonorrhoea or non-specific urethritis.

Sections stained for IgA showed a variable number of plasma cells scattered throughout the lamina propria with a tendency to clustering particularly in papillary projections; they fluoresced bright apple green against a weakly fluorescing background. There was almost

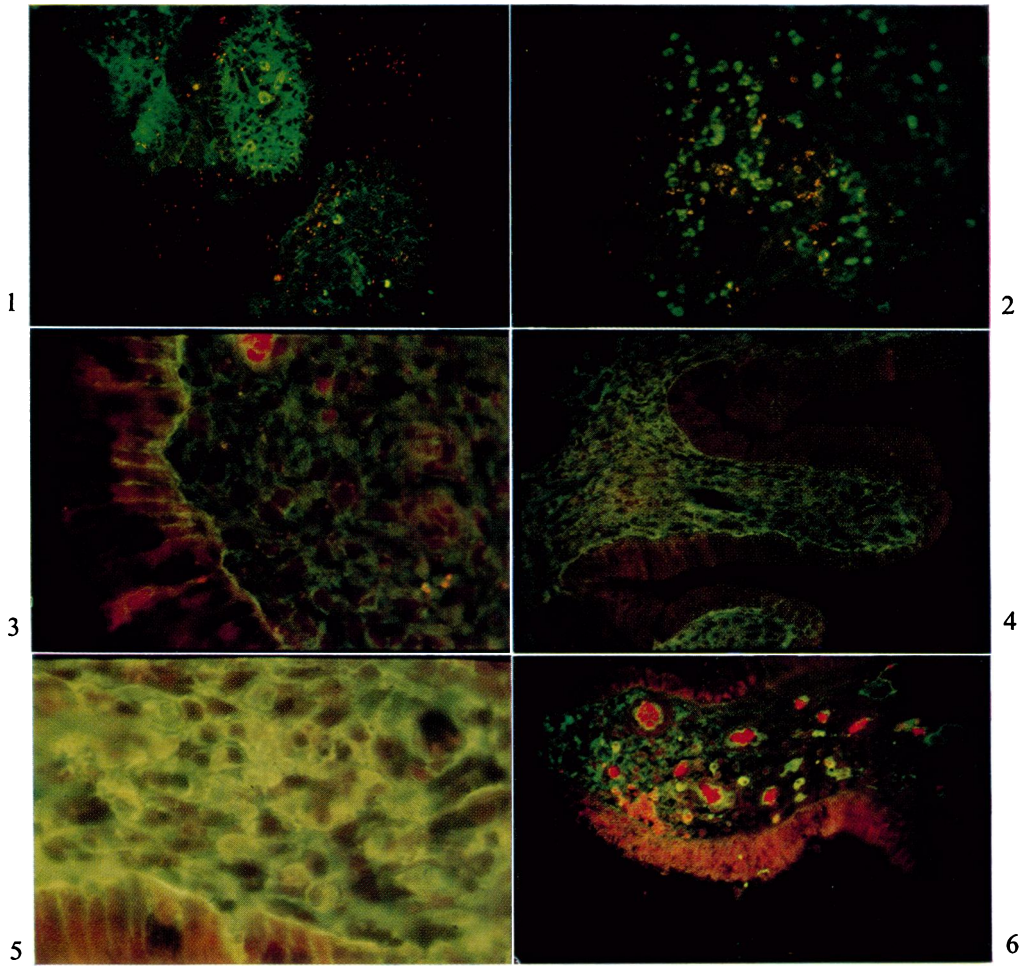

FIG. 1. Section of papillary projection stained with FITC anti-IgA. There is moderate stromal fluorescence, stronger fluorescence in the intercellular spaces of the epithelium and four fluorescing plasma cells. Original magnification × 64.

FIG. 2. Section stained with FITC anti-IgA. There is a large number (+ + +) of fluorescing plasma cells. Original magnification × 64.

FIG. 3. Section stained with FITC anti-IgG. There are no fluorescing plasma cells. There is some stromal fluorescence and strong fluorescence along the basement membrane and between the columnar epithelial cells. Original magnification × 240.

FIG. 4. Section stained with FITC anti-IgG. The fluorescing plasma cells are difficult to detect against the bright stromal fluorescence. Original magnification × 64.

FIG. 5. Same section as Fig. 4. At a higher magnification fluorescing plasma cells can be detected. Original magnification × 240.

FIG. 6. Section stained with FITC anti-IgM. Most fluorescence is associated with blood vessels. Only five fluorescing plasma cells are present. Original magnification × 64.

(*Facing p.* 221)

invariably fluorescence along the basement membrane and between the epithelial cells (Figs 1 and 2).

The IgG stain produced strong diffuse fluorescence in the stroma and more intense fluorescence along the basement membrane and between the columnar epithelial cells (Fig. 3). At low power magnification it was difficult to detect fluorescing plasma cells against the bright background. The problem was partially resolved by studying sections at higher power, but even so IgG-containing plasma cells appeared to stain less strongly than the other two classes and it was difficult to assess their number (Figs 4 and 5).

IgM was largely confined to the walls and lumen of blood vessels with minimal stromal fluorescence but some specimens showed moderate numbers of fluorescing plasma cells and occasionally there was also IgM along the basement membrane and up the sides of epithelial cells (Fig. 6). In one specimen, IgA was completely absent, but this showed a maximal number of plasma cells of the IgM class; subsequent estimation of serum levels of immunoglobulins revealed IgA deficiency (serum IgA 30 mg/100 ml). The number of fluorescing plasma cells in each class of immunoglobulin is shown in Table 1 arranged according to the diagnosis. The group in which no abnormality was detected by conventional smears and cultures was sub-divided to separate those patients who attended solely as contacts of gonorrhoea or non-specific urethritis.

DISCUSSION

Our findings show a clear relationship between plasma cell infiltration of the endocervical sub-mucosa and the presence of proven or probable local infection. Those patients with no abnormality and those attending solely as contacts of non-specific urethritis, with one exception in each group, have little or no evidence of local immunoglobulin production; either an undiagnosed infection, such as herpetic cervicitis, or another antigenic stimulus may explain the exceptions. By contrast, large numbers of plasma cells were present in cases of gonorrhoea and trichomoniasis. There was no apparent relationship with the menstrual cycle, oral contraception or cervical erosion. *N. gonorrhoeae* is known to invade mucus-secreting columnar epithelium, but *T. vaginalis* is not found in cervical mucus in cases of vaginal infestation; this could be a consequence of efficient local antibody production, and it is noteworthy that IgM is most prominent in this condition. Likewise, the proliferation of plasma cells seen in known female contacts of male patients with gonorrhoea from whom the organism was not isolated despite three attempts at weekly intervals, provides evidence of a killing effect by local immunity. On the other hand, there appeared to be no reaction in the endocervix to contact with non-specific urethritis, so that in this respect the behaviour of the aetiological agent does not correspond with *N. gonorrhoeae*. The effect of *C. albicans* was variable, which is consistent with its role as a facultative vaginal pathogen and its predilection for squamous epithelium.

Immunoglobulin production in the endocervical sub-mucosa might be considered as a mechanism required to prevent pathogenic invasion of the uterus and tubes. The incidence of acute salpingitis in our series is low, but both cases studied, one before and one 3 weeks after treatment showed no plasma cell infiltration, whereas we have found abundant plasma cells 3 weeks after successful treatment of cervical gonorrhoea.

Our observations in respect of gonorrhoea accord with the clinical difficulty experienced in diagnosing the condition in women (Catterall, 1970); organisms tend to decline in

TABLE 1. Number of plasma cells of the IgA, IgG or IgM class, assessed on a three point scale, in sections of endocervix of patients with and without local infection

Class of plasma cell:		Patient No.								
No abnormality diagnosed		63	74	71	82	10	41	47	75	
	IgA	++	+	±	±	−	−	−	−	
	IgG	++	−	−	−	−	−	−	−	
	IgM	−	−	−	−	−	−	−	−	
No abnormality diagnosed:		61	88	24	29	75	19	21		
contact of non-specific	IgA	+++	±	−	−	−	−	−		
urethritis	IgG	++	−	−	−	−	−	−		
	IgM	−	−	−	−	−	−	−		
No abnormality diagnosed:		66	96	76						
contact of gonorrhoea	IgA	+++	++	+++						
	IgG	++	++	+						
	IgM	±	±	−						
Gonorrhoea		16	102	92	62	15	90			
	IgA	+++	+++	+++	++	++	+			
	IgG	+++	++	++	−	−	−			
	IgM	±	+	−	±	−	−			
Vaginal trichomoniasis		60	32	31	25	50	84	86	39	
	IgA	+++	++	++	++	++	++	+++	+	
	IgG	+++	++	++	+	±	±	−	−	
	IgM	±	++	−	+	++	++	−	−	
Vaginal cardidosis		13	38	43	99	97	101	30	33	78
	IgA	++	++	++	±	+	+	−	−	−
	IgG	++	±	±	±	+	±	−	−	−
	IgM	±	±	−	±	−	−	−	−	−

number with the passage of time and the development of immunity. The present findings indicate that natural resolution may take place. Nevertheless, there is at present no alternative to repeated bacteriological examination to verify this. Isolation of *N. gonorrhoeae* by culture after 2 or 3 weeks would suggest that immunity has not been acquired and that chronic infection is established.

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REFERENCES

ABRAMS, L.D. (1958) A pleural-biopsy punch. *Lancet*, **i**, 30.

BATTY, I. & WARRACK, G.H. (1955) Local antibody production in the mammary gland, spleen, uterus, vagina and appendix of the rabbit. *J. Path. Bact.* **70**, 355.

BELL, E.B. & WOLF, B. (1967) Antibody synthesis *in vitro* by the rabbit vagina against diphtheria toxoid. *Nature (Lond.)*, **214**, 423.

CATTERALL, R.D. (1970) Diagnosis of vaginal discharge. *Brit. J. vener. Dis.* **46**, 122.

EIDELMAN, S. & DAVIS, S.D. (1968) Immunoglobulin content of intestinal mucosal plasma-cells in ataxia telangiectasia. *Lancet*, **i**, 884.

ELSTEIN, M. & POLLARD, A.C. (1968) Proteins of cervical mucus. *Nature (Lond.)*, **219**, 612.

HERVÉ, R., ROBEY, M. & SERGENT, P. (1965) Analyse immuno-électrophorétique du mucus cervical. Recherche d'un test d'ovulation. *Gynéc. Obstét.* **64**, 347.

HULKA, J.F. & OMRAN, K.F. (1969) The uterine cervix as a potential local antibody secretor. *Amer. J. Obstet. & Gynec.* **104**, 440.

MASSON, P.L. & FERIN, J. (1969) Les constituants protéiques du mucus du col. *Gynéc. Obstét.* **68**, 419.

MASSON, P.L., HEREMANS, J.F. & FERIN, J. (1968) Presence of an iron-binding protein (lactoferrin) in the genital tract of the human female. I. Its immunohistochemical localisation in the endometrium. *Fert. Steril.* **19**, 679.

MOGHISSI, M.D. & NEUHAUS, O.W. (1962) Composition and properties of human cervical mucus. II. Immunoelectrophoretic studies of the proteins. *Amer. J. Obstet. & Gynec.* **83**, 149.

PARISH, W.E., CARRON-BROWN, J.A. & RICHARDS, C.B. (1967) The detection of antibodies to spermatozoa and to blood group antigens in cervical mucus. *J. Reprod. Fert.* **13**, 469.

PIERCE, A.E. (1947) The demonstration of an agglutinin to *Trichomonas foetus* in the vaginal discharge of infected heifers. *J. comp. Path.* **57**, 84

SCHUMACHER, G.F.B., STRAUSS, E.K. & WIED, G.L. (1965) Serum proteins in cervical mucus. *Amer. J. Obstet. Gynec.* **91**, 1035.

SOLISH, G.I., GERSHOWITZ, H. & BEHRMAN, S.J. (1961) Occurrence and titre of isohemagglutinins in the secretions of the human uterine cervix. *Proc. Soc. exp. Biol. (N.Y.)* **108**, 645.

STRAUS, E.K. (1961) Occurrence of antibody in human vaginal mucus. *Proc. Soc. exp. Biol. (N.Y.)* **106**, 617.

STRAUS, E.K. (1965) Sperm immobilization in the human vagina by induced mucoantibody. *Fert. Steril.* **16**, 346

TOURVILLE, D.R., OGRA, S.S., LIPPES, J. & TOMASI, T.B. (1970) The human female reproductive tract: immunohistochemical localization of γA, γG, γM, secretory 'piece', and lactoferrin. *Amer. J. Obstet. Gynec.* **108**, 1102.