Case Reports

Gonadal Dysgenesis and Ulcerative Colitis

A Case Report with Clinical, Cytogenetic, and Post-mortem Studies

E. D. WILLIAMS,* E. ENGEL,† PRISCILLA D. TAFT, AND ANNE P. FORBES

From the Departments of Pathology and Medicine of Harvard Medical School, and the James Homer Wright Pathology Laboratories and the Medical Services (Thyroid and Endocrine Units) of the Massachusetts General Hospital, Massachusetts, U.S.A.

In recent publications (Forbes and Engel, 1963; Williams, Engel, and Forbes, 1964) we have described the high incidence of diabetes and thyroiditis in a series of cases of gonadal dysgenesis and in their close relatives, and have pointed out that a number of diseases in which an auto-immune mechanism has been suspected have occurred in association with this chromosomal disorder. In these reports no conclusions were drawn as to the cause of this association, though the possibility was suggested that the presence of auto-antibodies in the parents might lead to chromosomal abnormalities in the gametes or zygotes. Another possibility considered was that the chromosomal abnormalities in themselves led to a greater liability to auto-immune disease. The present report describes a patient with gonadal dysgenesis complicated by ulcerative colitis, who was studied clinically and cytogenetically, at the time of operation upon the colon and finally at necropsy. The findings suggested a third mechanism which could explain the development of auto-immune disease in some of these patients.

Case Report

Clinical History. A female, born in 1915, first entered the Massachussetts General Hospital in 1939 for recurrent attacks of abdominal pain. She was one of nine children. Her mother, 35 years old at her birth, died at age 68 of a 'coronary'. The father died at age 35 of 'Bright's disease'. One brother aged 45 died of a 'coronary', another at 46 of 'rheumatic heart disease'. One sister was 'born blind'. The patient had always been small and had never developed sexually. She had

been treated for purulent otitis media, for compound myopic astigmatism, for strabismus, and for a pterygium of the left eye. At the age of 25 she was 56·5 in. (143 cm.) tall and weighed 74 lb. (33·5 kg.). There was no webbing of the neck and the hair line was normal. The ears, however, were low in position and the external ear was malformed. Cubitus valgus was present. The hands and feet were long and thin. There were several pigmented naevi. Laparotomy, performed for signs of intestinal obstruction, revealed adhesions to the bowel, many calcified mesenteric lymph nodes, and, by palpation, 'very small' ovaries. Replacement therapy with oestrogen, in doses sufficient to cause secondary sexual development and regular withdrawal bleeding, was given from this time on. At the age of 40 the patient was readmitted for severe ulcerative colitis requiring corticotrophin and corticosteroid therapy. A 'Diagnex' tubeless test meal on this admission revealed no free gastric acid. The blood pressure was 175/125 mm.Hg. Steroids were gradually withdrawn during the next two years and the symptoms of colitis remained mild and controllable by antispasmodics. A tanned red cell test for circulating antibody to thyroglobulin carried out after stopping steroid treatment showed a titre of 1 : 20. Asymptomatic hypertension persisted. At the age of 47 severe bloody diarrhoea recurred and was uncontrollable by corticosteroid therapy. A colectomy was performed. Multiple transfusion and infusions of pressor amines were required to combat hypotension during anaesthesia, but thereafter convalescence appeared uneventful. However, on the eighth post-operative day the patient suddenly cried out, complaining of stabbing substernal pain, and expired within a few minutes.

Cytogenetic Findings. These are summarized in the Table and demonstrate the presence of mosaicism in this patient. 12 cells with 45 chromosomes were analysed; these originated from peripheral blood (9), bowel serosa (2), and skin (1): in each case a medium-sized chromosome was missing, and taken in conjunction with the absence of drumsticks and the low percentage of buccal mucosa cells with sex chromatin, these cells were interpreted as showing an XO chromosomal
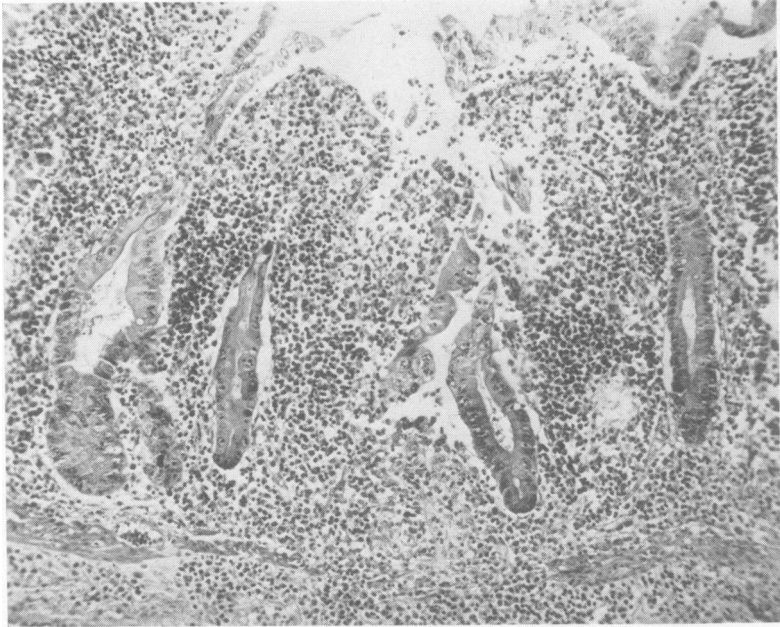
FIG. 1. Section of colon from operation specimen, showing the inflammation and mucosal ulceration. (H. and E. × 107.)
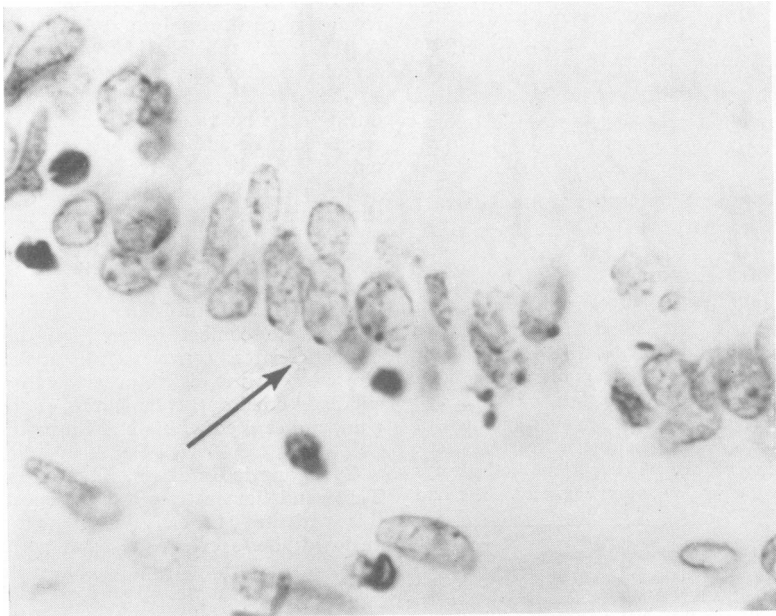

FIG. 2. Feulgen preparation of colon, demonstrating sex chromatin in several mucosal cells. (× 1290.)

TABLE

SUMMARY OF CYTOGENETIC DATA

Tissue	Sex Chromatin	No. of Chromosomes				
		44	45	46	47	Total
Blood	0/500 (drumsticks)	*6	27	1	—	34
Skin fibroblasts	12% {a	—	7	13	1	21
{b		*2	14	41	—	57
Buccal mucosa	14·5%	—	—	—	—	—
Bowel serosa	—	—	3	17	—	20
Bowel mucosa	33%	—	—	—	—	—
		8	51	72	1	132

* Probably due to loss during preparation.

constitution. 7 cells with 46 chromosomes were analysed; 2 from bowel serosa and 5 from skin; in each case one chromosome from the 6-X-12 group was missing, and an additional chromosome was present of the same size as autosome No. 3. This was interpreted as a presumptive isochromosome for the long arm of the X chromosome. Skin cultures were given a pulse exposure to tritiated thymidine, and of 72 metaphases examined after autoradiography 7 with 46 chromosomes showed heavy labelling of only one chromosome. Three of these were analysed, and in each case the labelled chromosome was of the same size as autosome No. 3. This was taken as further evidence that this was an isochromosome for the long arm of the X chromosome.

Histological Findings. The colon resected at operation showed the typical features of ulcerative colitis, with widespread shallow ulceration seen grossly, and crypt abscesses and mucosal ulceration present on microscopical examination (Fig. 1). Sections of the mucosa were stained with the Feulgen technique, and counts by different observers gave figures of 32 and 34%, respectively, of epithelial cells containing sex chromatin (Fig. 2).

Necropsy. At necropsy there was very severe atherosclerosis of the abdominal aorta and the coronary arteries, with great reduction in lumen of the anterior descending branch of the left coronary and complete occlusion at one point of the right coronary artery. The heart showed the scar of an old infarction in the anterior wall of the left ventricle, and an area of recent infarction in the lower intraventricular septum. The lungs showed extreme oedema. The small bowel ended in an ileostomy, but was otherwise normal. The terminal portion of sigmoid colon was uninvolved by disease. The liver showed some portal inflammatory infiltrate with slight fibrosis. A little fatty change was present. The vagina, cervix, and uterus appeared normal apart from the presence of a small endometrial polyp. The uterus measured 7 × 4 × 3 cm. The fallopian tubes were of normal length, but narrow; at the site of the ovary on each side was an ill-defined white streak about 3 cm. in length and up to 3 mm. in width. Histological study of these streaks revealed loose

vascular connective tissue with a zone of condensation of mesodermal tissue, producing the histological picture of ovarian stroma. No germ cells could be identified and no hilus cells were seen. The fallopian tubes showed poorly convoluted epithelium and a thin muscular wall. Kidneys, ureter, and bladder were normal except for the presence of a small number of foci of acute pyelonephritis in the upper pole of the left kidney. The adrenals (11 g. together) and thyroid (10 g.) were small, but grossly normal. A few foci of lymphocytic infiltration were present in the thyroid. The pituitary weighed 476 mg. and, *microscopically*, showed a normal or slightly increased number of acidophils. The basophil series and the pars nervosa were unremarkable. Four parathyroids of normal size were found; microscopically there was a slight increase in number of water-clear cells. In the central nervous system there was evidence of fairly recent cerebral softening in the right parieto-occipital region. Chronic otitis media was present bilaterally, with an irregular calcified cholesteatoma 1 cm. in diameter projecting into the cranial cavity on the left.

Discussion

This case brings out a number of points in relation to gonadal dysgenesis. Evidence of an old cardiac infarction was found, and severe atherosclerosis with coronary heart disease was present, despite long-continued oestrogen therapy. The cerebral softening is presumably related to cerebral arterial atherosclerosis and a period of hypotension at the time of operation.

In view of the sex difference in prevalence of atherosclerosis and the suggested role of hormones, the findings in this patient who had received adequate oestrogen replacement from the age of 25 stress the importance of genetic factors.

Deafness appears to be common in patients with gonadal dysgenesis, and this is the fourth known case of cholesteatoma among the 41 cases of the Massachusetts General Hospital series. Deafness and cholesteatoma were also noted by Lindsten (1963). It seems likely that some structural anomaly of the middle ear is present in these cases, rendering them particularly susceptible to repeated otitis media, and in some cases to cholesteatoma.

The major point of interest in this case is the occurrence of ulcerative colitis. Evidence has accumulated recently that this disease is associated with the presence of circulating antibodies to colonic mucosa (Broberger and Perlmann, 1959; Kraft, Bregman, and Kirsner, 1962), and this case is one of several examples of an auto-immune disease occurring in gonadal dysgenesis. The cytogenetic studies suggested that the patient was XO/X iso X mosaic. The great majority of the cells, presumed to be lymphocytes, grown from

the blood contained 45 chromosomes and showed the XO karyotype seen in typical Turner's syndrome. None of the 500 leucocytes examined contained drumsticks. However 13–16% of buccal mucosa cells were found to contain Barr bodies, indicating the presence of mosaicism. This was confirmed when dividing cells were cultured from tissues obtained at operation and cells with 46/X iso X karyotype were identified. These findings suggest that all the white blood cells were 45/XO, while the buccal mucosa, skin fibroblasts, and bowel serosa were composed of both 45/XO and 46/X iso X cells. The proportion of colonic epithelial cells showing prominent sex chromatin was in the normal female range. No normal 46/XX karyotypes were found in any cell analysed, and it seems likely that the bowel mucosa cells were 46/X iso X. In this case, therefore, we have evidence that the cells affected by presumed auto-immune disease are cytogenetically different from the antibody producing cells. This raises the possibility that the cytogenetic differences may be causally related to the development of the disease.

It does not follow that because the morphological difference between the two cell lines, X iso X and XO, is obvious, the functional and antigenic differences are necessarily remarkable. There is increasing evidence that one X chromosome is inactivated and forms the Barr body. According to the Lyon hypothesis (1962), inactivation is random and takes place during early embryonic development. Recent studies on females heterozygous for two types of glucose-6-phosphate dehydrogenase have confirmed at least partial inactivation of one X chromosome (Davidson, Nitowsky, and Childs, 1963). However, studies of several cases with an X iso X karyotype have led to the conclusion that inactivation under these circumstances is not random; when an isochromosome X is present it seems to be invariably involved in Barr body formation (Muldal, Gilbert, Lajtha, Lindsten, Rowley, and Fraccaro, 1963; Miller, Mukherjee, Bader, and Christakos, 1963). Autoradiographic studies, after exposing cell cultures from this case to tritiated thymidine, have demonstrated that the late-labelling chromosome is not the normal X, and provide further supporting evidence for the uniform inactivation of the iso X chromosome. If this sequestered chromosome plays no part in controlling protein production, then antigenic differences between the X iso X cells and the XO cells would be unlikely. However, we would like to point out that the uniform inactivation of one X chromosome creates a most unusual situation. In the normal female sequestra-

tion is random, so that the body has been exposed to the proteins coded by both paternal and maternal X chromosome, with the normal development of immune tolerance. In a patient with uniform sequestration of one X chromosome, tolerance will have been developed to the proteins coded by the active X chromosome. If in such a patient all or part of some of the sequestered X chromosomes acquired the property of coding protein synthesis, such proteins would be 'new' to the individual.

The mechanisms that lead to inactivation are not known, and reactivation or 'delyonization' of an inactivated X chromosome is a theoretical possibility. Such a reactivation could occur as a result of failure of the normal controls which ensure that the progeny of one cell inactivate the same X as the parent cell, or there could be partial reactivation of an X chromosome in intermitotic nuclei. Widespread complete reactivation in intermitotic nuclei seems unlikely, as neither Lennox (1956) nor Sutter (1960) found an appreciable drop in Barr body count with increasing age. Neither mechanism would be likely to lead to any trouble in a normal female, but in the individual with uniform sequestration of one X chromosome the resultant protein could be antigenic.

To attribute auto-immune disease affecting a whole organ to the production of an antigenic protein by some of the cells would imply either cross-reaction between that protein and the variant coded by the other X chromosome, or that the state of immune tolerance to one protein can be disrupted by a structurally similar antigen. Weigle (1963) has some evidence suggesting that either mechanism may happen: antibody produced by rabbits to chemically altered thyroglobulin cross-reacted with native thyroglobulin; and injections of altered bovine serum albumin ended a state of tolerance for the unaltered protein.

In all 4 reported cases of Hashimoto's disease and gonadal dysgenesis an X iso X karyotype was found (Sparkes and Motulsky, 1963; Williams et al., 1964), and in the case of ulcerative colitis and gonadal dysgenesis reported here the colonic mucosa is almost certainly X iso X, while the blood cells are XO We consider that the reactivation of at least part of the uniformly sequestered X chromosome is a plausible hypothesis to explain the development of auto-immune disease in these cases.

This hypothesis does not explain the incidence of thyroid antibodies in XO cases of gonadal dysgenesis; though the possibility of a hidden tissue mosaicism cannot be excluded. It is worth noting that uniform sequestration of one X could

well occur by chance in every cell of a small organ, such as the thyroid, in a normal female. The number of precursor thyroid cells in the 14-day embryo, the period around which 'lyonization' is said to occur, is probably extremely small. In addition, uniform inactivation might result from minor X chromosome anomalies. It is, therefore, possible that some cases of auto-immune disease in females could be explained by reactivation of a segregated X chromosome.

Summary

A case of ulcerative colitis occurring in a patient with gonadal dysgenesis is described. Full clinical, cytological, and pathological investigations were carried out, including a necropsy.

It was established that the patient was an XO/X iso X mosaic, with strong evidence to suggest that the blood cells were XO, and the colonic mucosal cells X iso X.

The frequency of the X iso X karyotype in cases of gonadal dysgenesis with auto-immune disease is discussed, and a possible mechanism by which this could arise is presented.

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