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ANTIBODY TO THE OXYPHIL CELLS OF THE HUMAN PARATHYROID IN IDIOPATHIC HYPOPARATHYROIDISM

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SUMMARY

IgG antibody to parathyroid oxyphil cells is described in the serum of one of nine patients with idiopathic hypoparathyroidism. This serum also reacted with the chief cells of the parathyroid. Whether other sera from the patients with hypoparathyroidism contained antibody to chief cells in low titre was uncertain.

INTRODUCTION

Idiopathic hypoparathyroidism belongs to the 'club' of organ-specific autoimmune diseases along with the thyroid autoimmune diseases (thyrotoxicosis, Hashimoto goitre, primary atrophic hypothyroidism), idiopathic Addison's disease, Addisonian pernicious anaemia and certain cases of premature ovarian failure (Irvine, 1965; Blizzard, Chee & Davis, 1967; Irvine, Stewart & Scarth, 1967; Irvine *et al.*, 1968). Blizzard, Chee & Davis (1966) have described the presence of parathyroid antibodies as detected by the indirect immunofluorescent technique in 38% of seventy-four patients with idiopathic hypoparathyroidism, 26% of ninety-two patients with idiopathic Addison's disease, 12% of forty-nine patients with Hashimoto thyroiditis and in 6% of 245 control patients. No distinction was made between antibody to the chief (principal) cells or to the oxyphil cells of the parathyroid. The present paper describes antibody to the oxyphil cells and discusses some of the difficulties in the detection of antibody to the chief cells of the parathyroid.

MATERIALS AND METHODS

A limited number of unfixed frozen sections were obtained from a small parathyroid adenoma from a female patient aged 42 years with hyperparathyroidism. Immediately after the removal of the adenoma from the patient, the tissue was rapidly frozen and the sections prepared. Parathyroid tissue was also obtained at routine post mortem from a male subject aged 50 years with no clinical evidence of parathyroid disorder. The sections were used in the

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indirect immunofluorescent antibody technique. The sera from nine patients with idiopathic hypoparathyroidism and from nine control subjects matched for age and sex were tested. Horse anti-human IgG conjugated with fluorescein isothiocyanate isomer I (British Drug Houses, England) with the fluorescein/protein ratio of 0·67 was used in a dilution of 1:6. The washing fluid in the immunofluorescent technique was veronal buffer, pH 7·2. Ten per cent glycerol in veronal buffer was used to mount a coverslip. Full details of the preparation of the conjugate and of the immunofluorescent staining are given elsewhere (Irvine, Chan & Williamson, 1969b). The sections were examined under a Leitz orthoplan microscope with a HBO 200 W lamp, primary filter BG 12 (5 mm), Tiyoda oil immersion supra wide angle dark ground condenser with numerical aperture 1·20 and secondary filter 530. The results were read subjectively.

Complement fixation tests were done using a phosphate buffer extract of a second and larger parathyroid adenoma (adenoma II) that had been stored at −20°C for some months. None of the tissue from the second parathyroid adenoma had been snap frozen so that it was not suitable for immunofluorescence studies. The Takatsy microtitre technique (Irvine, 1966) was used with doubling dilutions of control and test sera and with dilutions of a veronal buffer extract of parathyroid adenoma II.

The parathyroid cryostat sections were also tested in the indirect immunofluorescent antibody technique with a serum (B.4320) known to contain a high titre of 'M' antibody (Doniach *et al.*, 1966). Control cryostat sections of rat kidney were included. Serum absorption experiments with a veronal buffer extract of rat liver were also carried out. One volume of rat liver was homogenized with 3 volumes veronal buffer (M.S.E. homogenizer). After centrifugation at 1750 *g* 10 min, the supernatant was used for absorption. One volume antigen and 1 volume serum were incubated for 3½ hr with continuous shaking at room temperature (Griffin Flask Shaker). After further centrifugation, the supernatant was used in complement fixation and immunofluorescence tests. Extracts of other tissues were used for absorption following the same procedure.

The hypoparathyroid and control sera were also tested against cryostat sections of human gastric mucosa, thyroid, adrenal, ovary and testis.

RESULTS

The serum of one patient (B.8106) with idiopathic hypoparathyroidism showed clear immunofluorescent staining of the oxyphil cells of the parathyroid adenoma I (Fig. 1a) and of normal parathyroid (Fig. 1b). The patient was from Puerto Rico and her serum was made available to me through the kindness of Dr Lillian Haddock from the University School of Medicine, Puerto Rico, and Dr Robert Blizzard, The Johns Hopkins Hospital, Baltimore. She is patient J in the paper by Irvine, Chan & Scarth (1969a), and is also referred to in Irvine (1969a). She developed idiopathic hypoparathyroidism at 2 years and idiopathic Addison's disease at 19 years.

Serum B.8106 gave a negative reaction in the indirect immunofluorescent test for 'M' antibodies (Doniach *et al.*, 1966) when tested against rat kidney, but as described in Irvine *et al.* (1969a) gave a positive reaction in the indirect immunofluorescent antibody technique with all three layers of human adrenal cortex, certain steroid-producing cells in human or rabbit ovary, interstitial cells of human or rabbit testis, rabbit spermatids and with placental trophoblasts. Her serum was also positive for antibody to human gastric parietal cell in the

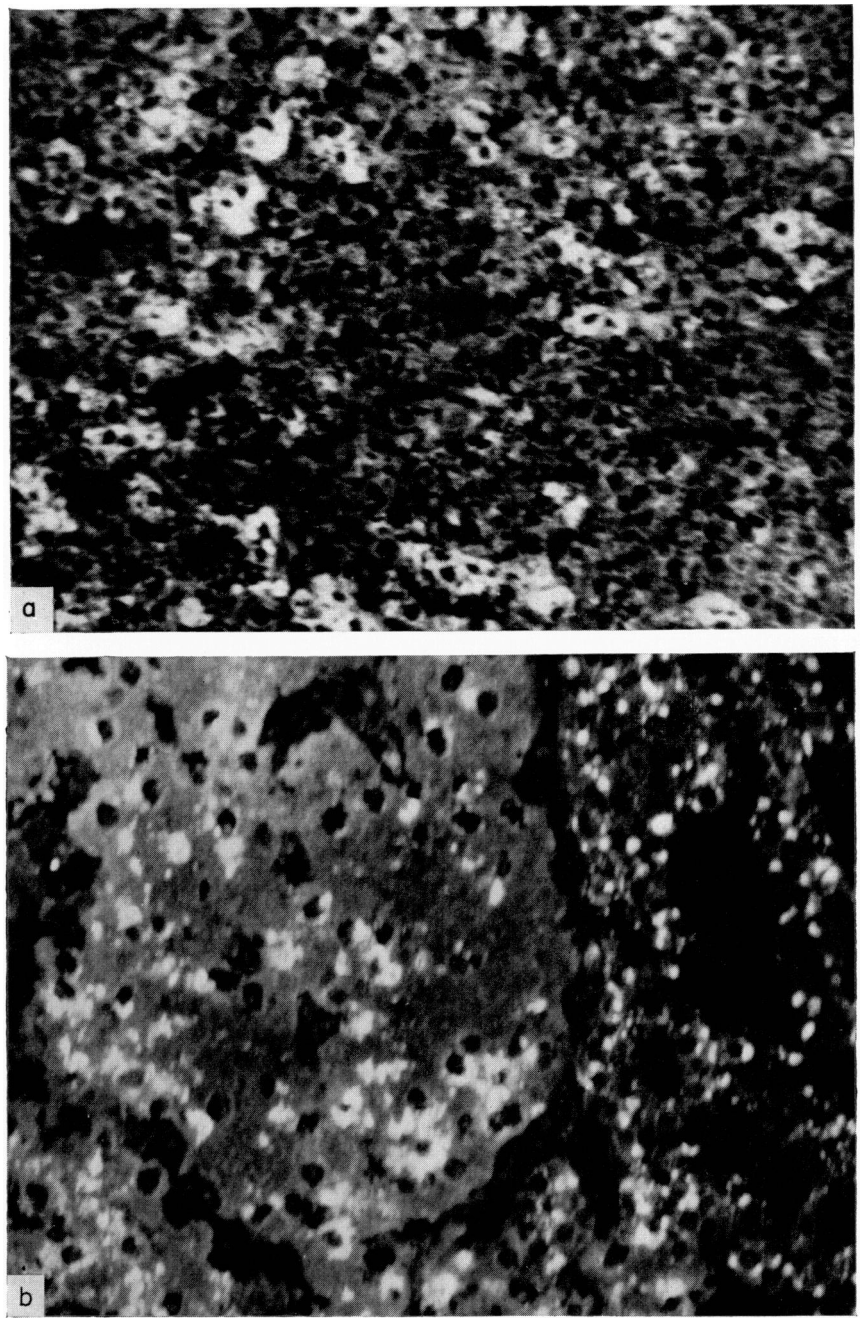

FIG. 1. (a) Positive reaction in the direct immunofluorescent antibody technique with the cytoplasm of scattered oxyphil cells in an unfixed cryostat section of a parathyroid adenoma using serum B.8106 from a patient with idiopathic hypoparathyroidism and anti-human IgG–FITC conjugate. The cytoplasm of the parathyroid chief cells is also considered to show positive immunofluorescent staining. Dark ground UV–blue light, × 185. (b) Same as (a) but with normal post-mortem parathyroid. In this instance the oxyphil cells are present in a discrete cluster. The bright globules are due to redish orange autofluorescence and not to the green fluorescence of the specific staining of the cytoplasm of the parathyroid cells. Dark ground Ultraviolet–blue light, × 400.

indirect immunofluorescent technique, but gave a negative immunofluorescence test for antibody to human thyroid epithelial cytoplasm.

Complement fixation tests with serum B.8106 were negative with rat liver and with human thyroid extracts, but positive with an extract of mucosa from the body of human stomach. The complement fixation tests with parathyroid adenoma II were equivocally positive on chessboard titration at a serum dilution of 1:4 and an antigen dilution of 1:4. Serum B.8106 was the only one to give even this degree of reaction in the chessboard titration with a buffered saline extract of parathyroid adenoma II out of the eighteen sera so tested.

Serum B.4320, which was known to contain high titres of 'M' antibody, was tested against cryostat sections of parathyroid adenoma I and of the normal parathyroid. Positive immunofluorescent staining of the cells with comparable distribution to the oxyphil cells stained by serum B.8106 was obtained and the fluorescence was of similar intensity. However, serum B.4320 gave an intense staining of the renal tubules and of the liver parenchyma when tested against rat kidney and liver sections, while serum B.8106 gave a negative reaction with these tissues.

The immunofluorescent staining of the oxyphil cells in the sections of normal parathyroid could not be absorbed out by rat liver extract. The titre of the IgG antibody to oxyphil cells was 1:16 before and after absorption. In contrast, the titre of serum B.4320 with the oxyphil cells in the normal parathyroid sections was reduced from 1:256 to 1:64 by prior absorption of this serum with liver extract.

Serum B.8106 also gave a distinct and consistent increase in the brightness of fluorescence of the chief cells in cryostat sections of parathyroid adenoma I and of the normal parathyroid. The titres of the reaction with the parathyroid chief cells given by serum B.8106 and by serum B.4320 before and after absorption with liver extract were comparable to those recorded, respectively, for the reaction with the parathyroid oxyphil cells. Three of the other eight sera from patients with idiopathic hypoparathyroidism and one of the nine control sera were also considered to give a positive indirect immunofluorescence test with the chief cells of parathyroid adenoma I but the results were not consistent when checked with sections of the normal parathyroid. The interpretation of a positive or negative immunofluorescence reaction with the chief cells presented some difficulty. This was because there was no clear contrast within the specimen itself as in the case of the oxyphil cells and it was necesary to assess whether the general degree of fluorescence in the cytoplasm of these cells in each section was greater or equal to that of the controls, there being some slight degree of background staining.

The control serum (B.4752) that appeared to give a positive indirect immunofluorescence test for the cytoplasm of the chief cells in parathyroid adenoma I was shown to contain antibody to human parietal cells and to human thyroid epithelial cytoplasm but was negative for 'M' antibodies and for antibodies to steroid-producing cells in the gonads and placenta. One other control serum gave a positive immunofluorescence test for gastric parietal cell antibody, but otherwise the control sera were all negative.

DISCUSSION

The occurrence of antibodies that are specific for one or each of the cell types in the parathyroid is what one would anticipate in the sera of at least a proportion of patients with idiopathic hypoparathyroidism. As already mentioned, this condition is associated clinically

with other disorders that are characterized by the formation of autoantibodies that are highly specific for components of individual tissues. The histology of idiopathic hypoparathyroidism is that of atrophy of the parathyroid cells together with lymphocytic infiltration (see Irvine, 1969b). Lupulescu *et al.* (1968) have demonstrated that repeated inocculation of homologous parathyroid tissue into dogs may induce isoimmune hypoparathyroidism with the characteristic biochemical and histopathological features and the presence of complement fixing parathyroid antibodies in low titres in the serum. The isoimmune hypoparathyroidism in dogs was similar to that described by Lupulescu *et al.* (1965) in rats.

Serum B.8106 which gave a positive immunofluorescence test with the oxyphil cells in parathyroid adenoma I and in the normal parathyroid also reacted positively with human gastric parietal cell cytoplasm and with steroid-producing cells in the gonads. The negative immunofluorescence reaction with rat kidney tubules and with rat liver sections and with human thyroid epithelial cytoplasm and the negative complement fixation tests with rat liver indicate that the positive immunofluorescence reaction with the oxyphil cells was not due to the presence of tissue non-specific 'M' antibodies. This is confirmed by the failure of liver extract to absorb the antibody reacting with parathyroid oxyphil cells. The distinction from 'M' antibodies is an important point to establish as the oxyphil cells are known to be rich in mitochondria and it has been demonstrated in this paper that 'M' antibodies can give positive immunofluorescence tests with parathyroid sections in the distribution of the oxyphil cells. Also in favour of the immunofluorescence reaction of serum B.8106 being specific for parathyroid is the observation that 'M' antibodies are rare in the organ-specific group of diseases (Doniach *et al.*, 1966).

The application of microphotometry (Irvine *et al.*, 1969b) may allow a more confident distinction to be made between positive and negative immunofluorescent reactions with the chief cells of parathyroid tissue. The findings in the limited series reported here are in keeping with those reported in a much larger series by Blizzard *et al.* (1966). However, in Blizzard's series it is anomalous, in view of the rarity of idiopathic hypoparathyroidism, that as many as 6% of this control subjects were positive for parathyroid antibodies presumably to chief cells. It is also anomalous that the suitability of parathyroid adenomata for fluorescence studies could not be predicted and that no confirmatory reports have so far appeared. Clearly the incidence and characterization of parathyroid antibodies requires further study. It is not possible on the present evidence to state whether the parathyroid oxyphil and chief cells have distinct autoantigens one from the other.

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