The immunological hazard of Cushing's syndrome

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British Medical Journal, 1975, 4, 678-680

Summary

A 24-year-old woman was found to have cryptococcal meningitis and Cushing's syndrome due to an adrenal adenoma. Her meningitis was successfully arrested with fluorouracil. Treatment with metyrapone decreased her cortisol production and produced clinical remission of Cushing's syndrome. On admission her peripheral T lymphocytes were few and hyporeactive. When the overproduction of cortisol ceased the numbers of T lymphocytes and their reactivity returned to normal and she developed in-vitro lymphocyte responsiveness to the cryptococci.

Introduction

Blood cortisol concentrations that remain raised for a long time have several noxious consequences, including suppression of the immune system.¹ The action of corticosteroids on white blood cells is complex. From experimental animal work we know that the cortical thymic lymphocytes are more sensitive to the lytic action of hydrocortisone than the more mature thymus medullary lymphocytes.² In man the peripheral thymus-derived (T) lymphocytes seem to be more sensitive than bone marrow lymphocytes,^{3,4} but the precise mode of action of high doses of corticosteroids in man is not known.

We report here on a patient with endogenous overproduction of hydrocortisone and its hazardous, but promptly reversible, effect on circulating T lymphocytes. This patient presented special clinical problems that cannot be created experimentally in man. Only two pathological conditions were present: high cortisol concentration and an infection that is seldom dangerous in man.

Case report

A 24-year-old woman was admitted to the department of infectious diseases, Danderyd Hospital, in December 1972 because of suspected meningitis. She had had oligomenorrhoea since December 1971 followed by amenorrhoea and periods of headache since September 1972. In the last two years she had successively gained weight and developed a rounded face, mild exophthalmus, and slightly increased hair growth on her face. In May-June 1972 she had travelled in Afghanistan.

On admission she had acute headache, nausea, fever (40°C), and rapidly progressing mental deterioration. Lumbar puncture disclosed $0.2 \text{ cells} \times 10^9/\text{l}$ ($200/\text{mm}^3$) in the clear cerebrospinal fluid (CSF) with equal proportions of lymphocytes and leucocytes. Protein concentration was 1.8 g/l and the ratio of blood: CSF glucose was 2:10. Other investigations showed: erythrocyte sedimentation rate 32 mm in one hour; white blood cell count $3.2 \times 10^9/\text{l}$ ($3200/\text{mm}^3$) with only 10% lymphocytes; normal serum electrophoresis, including γ -globulin;

normal serum electrolytes (sodium, potassium, calcium, magnesium, and phosphorus); and normal blood glucose. The chest x-ray film was normal and blood pressure was 110/80 mm Hg.

Using morphological criteria it was rapidly disclosed that her meningitis was caused by capsule-bearing *Cryptococcus neoformans*, as these organisms were abundantly found in the CSF pellet, where they were identified by phase-contrast microscopy. *C. neoformans* were also grown from the first two specimens of CSF. The patient was therefore immediately put on fluorouracil (Fluoro-uracil, Roche) by mouth in a dose of 250 mg/kg body weight divided into six doses per 24 hours. The concentration of fluorouracil four hours after a dose of 3 g was 60 mg/l in serum and 20 mg/l in CSF. Three days after fluorouracil treatment started her body temperature was normal. One week after treatment started the cryptococci could no longer be grown from the CSF, although they could still be morphologically identified.

As cryptococcal meningitis is an exceedingly rare disease in Sweden, fewer than 10 cases being known, we looked for predisposing diseases. The lymphopenia, amenorrhoea, and the patient's history pointed to Cushing's syndrome, and the change in her appearance was obvious on comparing photographs taken two years earlier with current ones. Absence of a diurnal rhythm of plasma cortisol and no suppression of plasma cortisol after 1 mg of dexamethasone⁵ supported the diagnosis. The patient was therefore transferred to the department of endocrinology and metabolism at Karolinska Hospital. Investigations showed poor suppressibility of plasma cortisol after dexamethasone 1 mg but an increase after an intravenous injection of 0.25 mg of tetracosactrin (synthetic ACTH). Because of the risk of activating her meningitis we did not perform extended suppression tests with high doses of dexamethasone,^{6,7} which are routine in the department. Plasma ACTH values, measured by radioimmunoassay, were 230-345 ng/l. These figures were within our normal range for the method. Further analysis showed urinary 17-ketosteroid values of 19.1-22.6 $\mu\text{mol}/24 \text{ h}$ (5.5-6.5 mg/24 h) and 17-ketogenic steroid values of 76.6-96.5 $\mu\text{mol}/24 \text{ h}$ (21.9-27.8 mg/24 h). Metyrapone 750 mg 6 times daily did not influence the urinary excretion of 17-ketogenic steroids. Plasma ACTH was unchanged when metyrapone or dexamethasone was given. Abdominal aortograms were normal as were x-ray films of the sella turcica. The visual fields were normal. Although an adrenal adenoma could not be verified by x-ray examination, it remained a possibility, mainly because of the findings at the metyrapone test.

We suspected that her high plasma cortisol level hampered her lymphocyte defence mechanisms against the fungus infection. We considered it necessary, however, to cure the meningitis before surgically treating her adrenals. Thus medical treatment of Cushing's syndrome was started with metyrapone 1250 mg/day while she was still on fluorouracil. Before the start of the metyrapone treatment the mean value of five determinations of plasma cortisol at even intervals over 24 hours was 635 nmol/l (23 $\mu\text{g}/100 \text{ ml}$), and after three days on metyrapone it was 331 nmol/l (12 $\mu\text{g}/100 \text{ ml}$). Her clinical signs of Cushing's syndrome disappeared rapidly and she resumed regular menstruation.

After stopping fluorouracil treatment her CSF returned slowly to normal, though a few cryptococci could still be histologically verified. She was operated on in August 1973, and an adrenal adenoma weighing 14 g was disclosed in her left adrenal gland. Her right adrenal was atrophied. Postoperatively the patient required replacement treatment with cortisone; this was discontinued two months after the operation. At the time of writing the patient was well and pregnant. In May 1974 her CSF was completely normal and free from cryptococci.

METHODS

Blood lymphocytes were purified by Böyum's⁸ method and the functional test was performed routinely.⁹ Values were expressed as logarithmic or arithmetic mean counts per 10^6 cells per minute. The tissue origins of blood lymphocytes were identified by the method of Jondal *et al.*¹⁰ The source of complement for the erythrocyte antibody complement (EAC) rosettes (bursa-equivalent (B) lymphocytes) was

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¹⁴C-incorporation (cpm/10⁶ lymphocytes) on day 5 in vitro of patient's (P) and control's (C) lymphocytes

Antigen added on day 0	Time after admission to hospital							
	3 weeks		11 weeks		21 weeks		60 weeks	
	P	C	P	C	P	C	P	C
Cryptococcus 200 µg*	1 400	2 400	2 650	3 400	90 500	1 200	16 950	5 300
Cryptococcus 2 µg*	1 850	3 000	2 000	3 800	23 650	1 800	39 750	3 450
PPD 100 µg	2 950	18 400	6 850	26 950	9 450	16 200	7 200	18 650
PPD 1 µg	1 400	64 450	3 900	37 100	4 700	44 750	7 250	31 600
None	950	2 900	2 000	2 850	4 200	3 000	2 950	3 800

*Amount of dry weight (freeze-dried material) in 0.1 ml of saline.

mouse serum from inbred A/Sn mice genetically lacking complement factor 5 and thus incapable of promoting lysis of antibody-coated target cells.

The phagocytic capacity of blood leucocytes was tested according to a method¹¹ which measures, in the presence of 10% normal human serum as a source of opsonin, the capacity of blood leucocytes to absorb as well as to kill live *Staphylococcus albus* organisms. Cells for this test were purified by sedimentation of whole blood in plasma gel.

ACTH in plasma was measured by a radioimmunoassay procedure described by Berson and Yalow.¹² Cortisol in plasma was determined according to the method of de Moor *et al.*¹³ as modified by Laurell.¹⁴ The urinary excretion of cortisol metabolites was measured as 17-ketogenic steroids as described by Appleby *et al.*¹⁵ The metyrapone stimulation test was performed by the method of Liddle *et al.*¹⁶ A short ACTH suppression test with dexamethasone, immediately followed by a single injection of synthetic tetracosactrin (Synacthen), was performed by the method of Thorén *et al.*¹⁷

RESULTS OF SPECIAL STUDIES

The lymphocyte function and the lymphocyte count of this patient were continuously followed and compared with those of a healthy 34-year-old man. The DNA-synthetic response of the patient's lymphocytes after the addition of phytohaemagglutinin (PHA) was drastically reduced on admission and remained so during initial treatment with fluorouracil. Soon after the start of treatment with metyrapone her lymphocytes regained reactivity to PHA (fig 1), and this normal reactivity was sustained throughout the test period (60 weeks). Her blood lymphocyte responsiveness to purified protein derivative of tuberculin (PPD) did not follow the same pattern (see table), although she would most probably have been immune to these antigens before falling ill, as virtually all young Swedes display immunity to these antigens as a result of compulsory immunisation with bacillus Calmette-Guérin vaccine in newborn infants.¹⁸ She did not regain immunity to these antigens after her plasma cortisol levels had been reduced with metyrapone. In contrast, her blood lymphocytes responded to crude water extracts of the cryptococci some time after the start of treatment with metyrapone, whereas the control lymphocytes never responded to these antigens (see table).

FIG 1—DNA-synthetic response of patient's (●) and control's (○) blood lymphocytes with (—) and without (---) addition of phytohaemagglutinin according to time and treatment.

As to the proportions of rosette-forming cells that were T cells or B cells, the directly rosette-forming cells comprised only 28% of the patient's lymphocytes on admission, as against 70% in the control (fig 2). The percentages of cells forming rosettes with red cells carrying activated complement were comparable in the patient and the control, which meant that only about half the patient's blood lymphocytes could be identified by these means on admission. After the start of treatment with metyrapone the proportion of directly rosette-forming lymphocytes rapidly returned to normal.

Phagocytic tests with blood leucocytes from the patient showed normal or even supranormal values from the time of admission to hospital.

FIG 2—Percentage of blood lymphocytes binding sheep red blood cells directly (—) and binding sheep red blood cells coated with rabbit IgM and mouse complements (---) from patient (●) and control (○) according to time and treatment.

Discussion

Follow-up of the lymphocyte functions of this patient, who was suffering from both cryptococcal meningitis and Cushing's syndrome on admission to hospital, showed the following main points.

Firstly, in Cushing's syndrome continuous overproduction of hydrocortisone results in, among other things, profound damage to circulating T cells. This was shown by the drastically reduced proportion of blood lymphocytes able to bind sheep red blood cells (SRBC) spontaneously (erythrocyte (E) rosettes) as well as by a considerably decreased capacity of blood lymphocytes to respond to PHA.

Secondly, B lymphocytes and leucocytes seem less sensitive to endogenous overproduction of glucocorticoids, since even before treatment of the Cushing's syndrome the proportion of blood lymphocytes binding SRBC carrying activated heterologous complement on their surface (EAC-rosettes) appeared normal, as did the phagocytic and bactericidal capacity of blood leucocytes. In addition, the γ -globulin level in the serum of this patient was normal throughout, suggesting an intact production of γ -globulin by mature B cells. As the lymphocyte count at admission was (low $3.2 \times 10^9/l$ ($3200/mm^3$), however, the absolute

number of B lymphocytes in untreated Cushing's syndrome may still be reduced.

Thirdly, after medical interference with the overproduction of cortisol in Cushing's syndrome there is a very prompt reappearance of PHA-sensitive as well as directly SRBC-binding lymphocytes in the blood, suggesting a reversible effect of steroids on T lymphocytes or their progenitors.

The data also suggested the following more tentative conclusions. The decrease of T lymphocyte responsiveness caused by Cushing's syndrome is probably of pathogenic importance in the development of the cryptococcal meningitis, since the arrest of glucocorticoid overproduction was followed by a specific in-vitro lymphocyte responsiveness to cryptococcal antigen that paralleled CSF clearance of the fungi (see table). The fact that the patient's lymphocytes did not spontaneously regain reactivity to PPD probably indicates that the T cells sensitised to these antigens were eliminated by the corticosteroids and that no new cells appeared in the circulation after the cortisol overproduction was arrested because no sensitising antigen was available.

On the basis of clinical evaluation and the fact that cryptococci could be cultivated only from the CSF before and within one week of treatment with fluorouracil, we feel that this drug in a dose of 250 mg/kg body weight⁻¹ day⁻¹ has been effective in arresting the proliferation and further spread of the fungi. We cannot explain why only half of the patient's lymphocytes carried B-cell and T-cell markers before her endocrine disorder was treated, whereas more than 95% carried one of the markers after her steroid production had become normal.

The assumption that a cortisol-producing adrenal adenoma was the probable cause of Cushing's syndrome in our patient was based on the lack of increment of 17-ketogenic steroids when metyrapone was given and the excellent result of treatment with this drug. The increase of plasma cortisol when ACTH was given is inconsistent with the diagnosis of adrenocortical cancer.

Patients with a unilateral adrenal tumour and overproduction of cortisol should have an atrophied adrenal gland on the contralateral side, and this was the case in our patient. The cause is a suppression of ACTH by cortisol produced from the tumour. We found ACTH values within the normal range, however, which should have prevented the atrophy of the contralateral gland. We therefore concluded that the immunoreactive ACTH

measured was probably biologically inactive, and the basal meningitis in this patient may possibly have stimulated the pituitary gland to release big ACTH. This has, however, never been described before. Big ACTH has identical immunoreactive characteristics to those of 1-39 ACTH but is biologically inactive.¹⁹⁻²¹

We thank Dr Sverker Bernander for making and providing the cryptococcal antigen and Dr Peter Unger, Stockholm Blood Centre, for providing facilities for making the phagocytic tests. All experiments with blood lymphocytes were carried out by Ulla Classon and Gun Stenman.

The study was supported by grants from the Swedish Medical Research Council (SB).

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Edinburgh Emergency Asthma Admission Service

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British Medical Journal, 1975, **4**, 680-682

Summary

In December 1968 an emergency service was set up in Edinburgh to enable patients with severe asthma to be admitted to hospital without delay. Up to 31 August 1975, 82 such patients had been admitted on 162 occasions, on 116 without the intervention of a general practitioner. The service is extended to patients particularly at risk of developing fatal asthma, and since it began no patient has died from asthma outside hospital. One patient,

however, died from tension pneumothorax that developed after admission. We believe that similar services should be available throughout Britain.

Introduction

Death from asthma may occur quickly and almost without warning. Speizer *et al*¹ investigated the increased mortality from asthma during the 1960s and found that out of 171 deaths 137 occurred suddenly and unexpectedly. Although the mortality from asthma is now lower than it was then, asthma remains an important cause of death. Cochrane and Clark² showed recently that many deaths occur before admission to hospital. A significant fall in mortality was reported by Jones³ when patients were admitted to a general intensive care area and treated by a medical team trained in the treatment of severe asthma. This reduction was achieved in patients referred to the intensive care area from the medical wards of the same hospital and other

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