Sensitivity of mononuclear leucocytes to glucocorticoids in elderly hip-fracture patients resistant to suppression of plasma cortisol by dexamethasone

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Abstract

Objective: Elderly women with proximal femur fracture show a prolonged increase in plasma cortisol, which could have undesirable catabolic effects. Suppression of cortisol by dexamethasone is impaired, suggesting resistance to glucocorticoid effects at feedback inhibitory sites. We therefore wished to find out whether peripheral glucocorticoid sensitivity is normal.

Design: Peripheral blood mononuclear leucocytes were used as a model tissue. Blood samples were taken from elderly women about 2 weeks after hip fracture and from elderly control women. Each patient was then given 1 mg dexamethasone at 2300 h followed by further sampling at 0800 and 1600 h the next day.

Methods: Glucocorticoid-receptor binding parameters were measured by incubating whole cells with [³H]dexamethasone for 2 h at 37°C. Inhibition of cell proliferation by dexamethasone was assessed by addition of [³H]thymidine to cells cultured for 65 h with concanavalin A. Cortisol and dexamethasone concentrations were measured in the dexamethasone suppression test.

Results: As expected, the hip-fracture patients had raised morning cortisol concentrations and impaired suppression by dexamethasone. The cells of the patients had similar numbers of glucocorticoid receptors to those of the control subjects but higher values for K_d (i.e. a lower binding affinity). The cells of the patients incorporated less [³H]thymidine than the control cells in the absence of dexamethasone. The percentage inhibition by a saturating concentration of dexamethasone was unchanged but the concentration giving half-maximal inhibition was decreased (sensitivity was increased) at the higher of the two concanavalin A concentrations used.

Conclusions: These experiments in mononuclear leucocytes give no evidence of peripheral resistance to glucocorticoids in hip-fracture patients with impaired suppression of cortisol by dexamethasone.

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Introduction

Elderly women with proximal femur fracture show a prolonged adrenocortical response to injury. By 2–3 weeks plasma cortisol is higher than in younger patients with injuries of similar or greater severity (1). It is also higher than in healthy elderly control subjects or geriatric patients receiving long-term care, and these differences continue for at least 8 weeks (2). The increase in cortisol is substantial. At about 2 weeks after injury, urinary free cortisol excretion is about three times higher than in healthy subjects (3), and indirect estimates suggest a similar increase in the plasma free cortisol concentration averaged over 24 h (R N Barton, J G Rose & M A Horan, unpublished observations).

Proximal femur fracture in old people is known to carry not only a substantial mortality (4) but also a high morbidity. Recovery is often slow and many patients fail to regain their previous level of independence (5). It is possible that cortisol contributes to this deterioration through its catabolic effects. Not only may it retard wound healing and fracture repair (6) but it would also be expected to increase net protein breakdown in uninjured muscle (7) and thereby hinder mobilisation in patients who already have poor muscle function. However, it is conceivable that such effects are less than expected because of glucocorticoid resistance. Hip-fracture patients usually show incomplete suppression of plasma cortisol by dexamethasone (2, 8). There is evidence (9) that the receptors responsible for this action are the same as the Type II or glucocorticoid

receptors (GRs) mediating peripheral glucocorticoid effects, including catabolic changes in muscle (10). Down-regulation of GRs by raised glucocorticoid concentrations is well established *in vitro* and is sometimes seen in man (11).

Because of the potential for inhibiting cortisol secretion therapeutically, it is important to establish whether elderly hip-fracture patients are resistant to glucocorticoids or whether they would be expected to suffer the usual deleterious consequences of persistently high cortisol concentrations. We therefore looked for biochemical evidence of glucocorticoid resistance in peripheral blood mononuclear leucocytes (PBMLs). This tissue would be involved in any immunosuppressive effects of cortisol, and its glucocorticoid sensitivity may reflect that of other tissues such as muscle. We measured the whole-cell binding characteristics of dexamethasone and a well-known glucocorticoid effect, inhibition of concanavalin A (ConA)-induced mitogenesis by dexamethasone (12, 13). We carried out overnight dexamethasone suppression tests in the same patients and included measurement of plasma dexamethasone, which many studies have shown to be a determinant, although not the only one, of the response to this test in non-suppressors (14).

Subjects and methods

Subjects and procedure

Studies were carried out in eight control women aged 63–85 years (median 74 years) and 15 injured women aged 67–93 years (median 81 years). The difference in ages was not statistically significant. The control women (Table 1) were not selected to be 'disease-free' but had only minor health problems and perceived their health to be good. The injured women had all sustained proximal femur fracture (eight extracapsular, seven intracapsular) which had been treated surgically (by internal fixation or hemiarthroplasty) within 72 h. One patient had in addition a Colles' fracture of the wrist. No patient had concomitant disease likely to affect adrenal function or was receiving drugs likely to do so. Studies were started 10–19 (median 14) days after injury.

On the first day of the study, a blood sample (40–80 ml) was taken from each subject at 1000–1130 h

with heparin as anticoagulant for separation of PBMLs, differential cell counting and measurement of plasma cortisol. At 2300 h on the same day the patients, but not the control subjects, received 1 mg dexamethasone (Organon, Cambridge, UK) orally. On the following day, at 0800 and 1600 h, further blood samples were taken from the patients for measurement of plasma cortisol and dexamethasone.

The studies were approved by the ethics committees of Salford and Stockport Health Authorities and all subjects gave their informed consent to the procedure.

Preparation of mononuclear leucocytes

Blood was diluted 1:1 with PBS (Flow Laboratories, Rickmansworth, Herts, UK; pH 7.4), layered on to Ficoll 400 solution (Lymphocyte Separation Medium; Flow Laboratories; medium/diluted blood, 1:2, v/v) and centrifuged at 1000 *g* for 45 min at 4 °C. The mononuclear-cell layer was taken and the cells were washed three times in PBS and resuspended in complete RPMI 1640 medium (Flow Laboratories), containing 10% (v/v) foetal calf serum (Globe Farm, Esher, Surrey, UK), 2 mmol/l glutamine (Flow Laboratories) and 5000 U/ml gentamicin (Sigma, Poole, Dorset, UK). In each preparation the ratio of monocytes to lymphocytes was measured by staining the former for non-specific esterase (15). Viability measured by exclusion of trypan blue (Flow Laboratories) was always greater than 95%.

Measurement of dexamethasone binding

Tubes each containing about 8×10^6 PBMLs suspended in complete RPMI 1640 medium were incubated with 1 nmol/l (37 nCi/ml) [$1,2,4\text{-}^3\text{H}$]dexamethasone (Amersham International, Amersham, Bucks, UK) with or without 14 or 2000 nmol/l unlabelled dexamethasone (Sigma) in a total volume of 1 ml for 2 h in a shaking waterbath at 37 °C. Except for single label-alone tubes in one patient and one control subject, all incubations were performed at least in duplicate; usually there were enough cells for more than six tubes, and more replicates were then included at 15 nmol/l than at the other two dexamethasone concentrations. At the end of the incubation, 2 ml ice-cold PBS was added and the

Table 1 Details of control subjects.

Subject	Age (years)	Medical conditions	Medications
1	76	Hypertension, Ménière's disease	Aspirin, cinnarazine
2	71	Mild osteoarthritis, mild hypertension	None
3	63	Mild osteoarthritis	None
4	83	Cystocele, osteoarthritis	Paracetamol
5	85	Hypertension, glaucoma, basal cell carcinoma	Atenolol, pilocarpine, bendrofluzide
6	81	Osteoarthritis	Paracetamol
7	68	Psoriasis, mild osteoarthritis, mild obesity	None
8	69	Mild hypertension	Amiloride, cyclopentiazide

suspension was centrifuged at 700 *g*; the cells were then washed twice in PBS. Scintillation counting in Opti-phase Hisafe II (LKB Scintillation Products, Loughborough, Leics, UK) was performed with a TriCarb 1500 liquid scintillation analyser (Packard, Pangbourne, Berks, UK) on the cells and supernatants apart from the last wash. The count rates were stable for at least 48 h. Internal standardisation with [³H]toluene (Packard) was used.

Values for the 'concentration' and dissociation constant (K_d) of high-affinity binding sites were calculated by the Woolf plot (16) from the bound/free ratios at dexamethasone concentrations of 1 and 15 nmol/l, corrected for non-specific binding by subtracting the mean bound/free ratio at 2000 nmol/l dexamethasone. The Woolf plot has theoretical advantages over the Scatchard plot (16), although the choice made little difference in the present studies because the bound/free ratios were very reproducible. Computer runs with large numbers of simulated data showed that the estimates of the binding parameters were unbiased and that the choice of concentrations was close to optimal.

Preliminary experiments established that the incubation time was adequate to achieve equilibrium, that the non-specific binding was non-saturable and that the binding parameters were unlikely to be affected by cortisol originally present in the blood.

Mitogenesis-inhibition assay

PBMLs (2×10^5 /well) suspended in complete RPMI 1640 medium (0.25 ml) were cultured in quadruplicate in flat-bottomed microtitre plates (Bibby; Philip Harris, Manchester, UK) in the presence of ConA (Sigma; 1 and 10 μ g/ml) and dexamethasone (0, 0.1, 1, 3, 10 and 100 nmol/l) at 37 °C in an atmosphere of 5% CO₂ for 65 h. [*methyl*-³H]Thymidine (Amersham International; 0.5 μ Ci/well) was then added to the cultures, and 5 h later the cells were transferred to filter paper with a cell harvester (Titertek; Flow Laboratories). The filters were placed in scintillation vials and soaked with 0.5 ml ethanol for 30 min before addition of Hisafe II and scintillation counting. The count rates were stable for at least 48 h. Cells from most subjects were incubated similarly with 3 and 30 μ g/ml ConA in the absence of dexamethasone to establish dose-response curves for the mitogen.

Measurement of plasma corticosteroid concentrations

Cortisol was determined with an RIA kit (Immuno-diagnostics Ltd, Boldon, Tyne and Wear, UK). Dexamethasone was measured by RIA with an antiserum kindly supplied by Professor P Vecsei (University of Heidelberg, Germany). Because this gave up to 0.4% cross-reactivity with cortisol, 80–90% of the cortisol

was first removed by incubating 0.5 ml plasma with 0.5 ml cortisol-free serum (Biogenesis, Bournemouth, Dorset, UK), to increase the amount bound to corticosteroid-binding globulin, and passing it rapidly through a Sep-Pak C₁₈ cartridge (Waters, Watford, Herts, UK) which adsorbed dexamethasone but not bound cortisol. The dexamethasone was eluted with methanol which was then evaporated to dryness in the assay tubes. Recovery was 94–98%. The RIA was performed overnight at room temperature with [1,2,4,6,7-³H]dexamethasone (Amersham) in 0.08 mol/l borate buffer, pH 8.0, and separation was with dextran-coated charcoal. A small correction (<10%) was made for the immunoreactivity due to residual cortisol, the quantity of which was measured by extraction of samples with added [³H]cortisol run with each assay.

Statistical analysis

With the exception of the data for [³H]thymidine incorporation in the absence of dexamethasone, for which a two-way analysis of variance was carried out on the logarithms (see Fig. 2), non-parametric statistics were used throughout. Differences between paired data were assessed with Wilcoxon's signed-rank test and differences between unpaired data with Wilcoxon's two-sample rank test (17). Correlations were tested by Kendall's rank correlation method (18).

Results

As expected (2, 8), the morning plasma cortisol concentration (in the blood sample from which cells were prepared) was higher in the injured patients than in the control subjects (Table 2). On the morning after administration of dexamethasone, the patients had a wide range of cortisol concentrations (Fig. 1). Although the control subjects were not given dexamethasone, the concentrations in the patients were all above the highest of the post-dexamethasone cortisol concentrations measured earlier by the same method in control subjects (2). Moreover, 12 of 14 patients were non-suppressors by the usual criterion of 0.138 μ mol/l (19). By the afternoon there was no consistent further change and 9 of 12 patients were non-suppressors. The plasma dexamethasone concentration also varied widely in the morning but, in contrast with the lack of change in cortisol, it had fallen by at least half in every patient in the afternoon (Fig. 1). The afternoon values correlated with the morning ones for both cortisol ($P < 0.01$) and dexamethasone ($P < 0.001$). At neither time was there a significant correlation between the cortisol and dexamethasone concentrations.

The differential cell count showed a marked fall in the number of lymphocytes and rise in the number of granulocytes in the injured patients (Table 2). The percentage of monocytes in the PBML preparation was considerably higher in the injured patients than in the

Table 2 Plasma cortisol concentration, blood cell counts, parameters for dexamethasone binding and parameters for inhibition of mitogenesis by dexamethasone in mononuclear leucocytes from elderly control women and women with proximal femur fracture. Results are given as median with range in parentheses.

	Control women	Injured women
Plasma cortisol ($\mu\text{mol/l}$)	0.34 (0.19–0.60)	0.55 (0.40–1.24) ^b
$10^{-9} \times$ cell count in blood (cells/l)*		
Lymphocytes	2.6 (1.6–3.6)	1.3 (0.8–2.3) ^a
Monocytes	0.6 (0.6–0.7)	0.7 (0.2–1.6)
Granulocytes	4.7 (3.0–5.7)	8.1 (4.9–17.6) ^b
Monocyte content of cell preparation (%)†	16 (13–33)	31 (22–43) ^c
[³ H]Dexamethasone binding		
Receptor content (no./cell)	6200 (5300–7400)	7100 (5000–8700)
K_d (nmol/l)	7.7 (7.0–8.3)	9.8 (7.3–15.5) ^c
[³ H]Thymidine incorporation with 1 $\mu\text{g/ml}$ ConA‡		
Maximal inhibition (%)	95 (90–99)	92 (78–99)
IC_{50} (nmol/l)	2.0 (0.4–4.0)	1.5 (0.1–4.4)
[³ H]Thymidine incorporation with 10 $\mu\text{g/ml}$ ConA‡		
Maximal inhibition (%)	60 (43–91)	69 (32–87)
IC_{50} (nmol/l)	7.7 (4.9–8.8)	3.8 (0.1–17.9) ^a

$n = 8$ for control women, $n = 15$ for injured women, except as follows: * $n = 4$ for controls, $n = 14$ for injured; † $n = 14$ for injured; ‡ $n = 12$ for injured; || could not be calculated for one subject. Differences from control women were significant at ^a $P < 0.02$, ^b $P < 0.01$, ^c $P < 0.001$ (Wilcoxon's two-sample rank test).

control subjects (Table 2). It did not differ from the percentage expected from the two cell counts in whole blood (Wilcoxon's signed-rank test).

Measurement of [³H]dexamethasone binding showed no difference in the number of GRs between PBMLs from injured patients and those from control subjects (Table 2). However, K_d was higher in the patients (Table 2), indicating a lower affinity. The two binding parameters did not correlate with each other in either group.

Figure 1 Plasma cortisol and dexamethasone concentrations at 0800 and 1600 h in elderly women with proximal femur fracture who had taken 1 mg dexamethasone at 2300 h on the previous day. The difference in concentration between the two times was not significant for cortisol but was significant at $P < 0.01$ for dexamethasone (Wilcoxon's signed-rank test). Note that two points at 0800 h (cortisol 0.14 and 0.15 $\mu\text{mol/l}$, dexamethasone 8.6 and 8.6 nmol/l) did not have accompanying 1600 h values.

In the absence of dexamethasone, ConA caused a dose-dependent increase in [³H]thymidine incorporation into cultured PBMLs (Fig. 2). At 1 $\mu\text{g/ml}$ ConA, incorporation was a median of 20× that in the absence of ConA (results not shown); peak stimulation was observed at 10 $\mu\text{g/ml}$. At all ConA concentrations, [³H]thymidine incorporation in the PBMLs

Figure 2 Relationship between [³H]thymidine ([³H]TdR) incorporation and ConA concentration in mononuclear leucocytes from control elderly women (○) and women after proximal femur fracture (●). Data are for 2×10^5 cells incubated with 0.5 μCi [³H]thymidine. Values are shown as geometric means with s.e.m. ranges; note that both axes are logarithmic. Only subjects with data at all four ConA concentrations are shown; $n = 5$ for control subjects, 11 for injured patients. The difference between the two groups was significant by two-way analysis of variance performed on the logarithms ($P < 0.025$).

Figure 3 Relationship between inhibition of [^3H]thymidine ([^3H]TdR) incorporation and dexamethasone concentration in mononuclear leucocytes from control elderly women and women after proximal femur fracture. ($\circ$) and ($\bullet$), control and injured women respectively with 1 $\mu\text{g/ml}$ ConA; (∇) and ($\blacktriangledown$), control and injured women respectively with 10 $\mu\text{g/ml}$ ConA. Values are shown as mean $\pm$ S.E.M.; $n=8$ for control subjects, 12 for injured patients. Statistical analysis of parameters calculated from the data is given in Table 2.

from hip-fracture patients was around half that in those from control subjects, leading to parallel concentration–response curves when the data were plotted as logarithms (Fig. 2).

ConA concentrations of 1 and 10 $\mu\text{g/ml}$ were chosen for studying the inhibitory effects of dexamethasone. At 100 nmol/l dexamethasone, GRs should have been close to saturation. Consequently we took inhibition at 100 nmol/l to be maximal and calculated the dexamethasone concentration giving half-maximal inhibition (IC_{50}) accordingly, using log-linear plots (Fig. 3) to interpolate between concentrations. With 1 $\mu\text{g/ml}$ ConA, the dose–response curves for the injured patients and control subjects were very similar (Fig. 3) and there was no difference in maximal percentage inhibition (which approached 100%) or IC_{50} between them (Table 2). With 10 $\mu\text{g/ml}$ ConA, maximal inhibition was less nearly complete and IC_{50} was higher. The curve was shifted to the left for the patients (Fig. 3) and the IC_{50} was lower than that for the controls; there was again no difference in maximal percentage inhibition (Table 2). For the patients, maximal inhibition correlated positively with baseline [^3H]thymidine incorporation (in the absence of dexamethasone) when the ConA concentration was 1 $\mu\text{g/ml}$ ($P<0.05$) but not when it was 10 $\mu\text{g/ml}$. At neither ConA concentration did IC_{50} correlate with baseline [^3H]thymidine incorporation or maximal inhibition. The values for maximal inhibition and IC_{50} at 10 $\mu\text{g/ml}$ ConA did not correlate with those at 1 $\mu\text{g/ml}$.

At neither ConA concentration did the parameters for inhibition of mitogenesis by dexamethasone correlate with the parameters for GR binding, i.e. there was no correlation between maximal percentage inhibition and the number of receptors per cell or between IC_{50}

and K_d . In absolute terms, at 1 $\mu\text{g/ml}$ ConA, IC_{50} was much lower than K_d ; at 10 $\mu\text{g/ml}$ the difference was narrowed for the injured patients and had disappeared for the control subjects (Table 2). At both ConA concentrations, IC_{50} was much more variable than K_d .

Discussion

This study was based on our previous observation of a raised cortisol concentration and impaired suppression by dexamethasone in elderly women with proximal femur fracture (2, 8). Both these findings have been confirmed (Table 2, Fig. 1). Most patients had post-dexamethasone cortisol concentrations above the criterion value of 0.138 $\mu\text{mol/l}$ (19). In contrast, healthy (screened) elderly women and geriatric patients receiving continuing care for chronic medical conditions all had concentrations well below this threshold when studied by the same method (2). We also measured the plasma dexamethasone concentrations. All were above the value of 6 nmol/l proposed as a threshold for adequate suppression in non-depressed subjects at 0800 h, and all but two (with values of 1.5 and 1.7 nmol/l) were above the proposed threshold of 2 nmol/l at 1600 h (20). Moreover, there was no significant correlation between the plasma cortisol and dexamethasone concentrations within the group of patients. We conclude that in hip-fracture patients, as in depressed patients (14) and elderly patients with depression or dementia (21), the impaired suppression of cortisol was not primarily due to abnormally rapid clearance of dexamethasone.

There is evidence that dexamethasone inhibits the hypothalamo–pituitary–adrenal (HPA) axis primarily at the pituitary level (9, 22; but see 23) and by a mechanism involving changes in transcription, although not necessarily of the gene responsible for adrenocorticotrophin synthesis (24). It is well known that feedback inhibition of the axis can be overridden by noxious stimuli and that this ability is a function of both the nature and strength of the stimulus (24). It is unlikely that the classical stimuli after injury, fluid loss and pain, are still present at 2 weeks in hip-fracture patients who have been treated by fluid replacement and surgery. Thus it is possible that resistance to dexamethasone reflects an impairment in the mechanism of suppression itself, either in the pituitary or at distant sites such as the hippocampus, where such an impairment would lead to an increase in vasopressin and possibly corticotrophin-releasing factor drive (25). There could be a decrease in the abundance of GR, for example by down-regulation (11). Alternatively, the ability of bound GR to affect gene transcription could be impaired. A possible cause is the presence of cytokines, which are another stimulus to the HPA axis (26). Cytokines and glucocorticoids can inhibit each other's effects by direct interactions between their transcription factors as well as at the genomic level (27). We have

found increased circulating concentrations of tumour necrosis factor receptor and interleukin-1 receptor antagonist 2 weeks after hip fracture (I P M O'Connell, R N Barton & M A Horan, unpublished observations), suggesting a generally raised level of cytokine activity. Thus in the present experiments it was necessary to measure both GR and a glucocorticoid effect to find out whether there was general resistance to glucocorticoids.

We used PBMLs as a model tissue in which to study the binding parameters of GRs and an effect known to be mediated by GRs (28), namely inhibition of lectin-induced mitogenesis. There was no difference between the patients and control subjects in either total GR-binding capacity or inhibition of [³H]thymidine incorporation by a near-saturating dexamethasone concentration (Table 2). However, the findings with regard to binding affinity and sensitivity to inhibition were not so consistent. The injured women had a higher K_d , i.e. a lower affinity of binding, than the control subjects (Table 2). However, there was no corresponding change in the IC_{50} for inhibition of [³H]thymidine incorporation; on the contrary, the IC_{50} at a ConA concentration of 10 µg/ml was lower in the patients than in the control subjects, while there was no difference in IC_{50} between the groups at 1 µg/ml ConA. Thus this assay tended to show a heightened sensitivity to glucocorticoids rather than a diminished one as the binding assay would predict.

These indices are influenced by a large number of factors. The exact meaning of K_d is not completely clear because the steroid-GR complex exists in several forms owing to activation and translocation to the nucleus (29); once activation has occurred, dissociation takes place via a different route (30). This heterogeneity is revealed by dissociation kinetics even though equilibrium studies show a single class of binding site (31). The sensitivity of [³H]thymidine incorporation to glucocorticoids depends on yet more factors since it decreases as the concentration of mitogen is raised from suboptimal to optimal (32), as our results confirm (Fig. 3). This additional complexity may account for the much greater variability of IC_{50} than of K_d (Table 2) and possibly also for the tendency of IC_{50} to be lower than K_d , as observed previously (33). Without a greater understanding of the control of these variables, interpretation of the opposite changes in K_d and IC_{50} is difficult. We can only conclude that there is no consistent impairment of sensitivity to glucocorticoids in elderly hip-fracture patients.

The comparisons in these assays could have been biased by two differences between the patients and control subjects. First, the fewer lymphocytes in the blood of the patients led to a higher monocyte content in the PBML preparation (Table 2). Since monocytes have around twice as many receptors per cell as lymphocytes (34), the difference in composition should have caused the patients to have a 10–20% higher GR content than

the controls. This probably accounted for the non-significant 15% difference observed (Table 2). There should have been no bias in the comparison of K_d , which is similar in lymphocytes and monocytes (34). Monocytes are known to influence proliferation of lymphocytes through production of cytokines such as interleukin-1 (35), and in theory the mitogenesis-inhibition assay could have been affected by the difference in composition of the cell preparations. Addition of monocytes to lymphocytes greatly desensitises them to inhibition of [³H]thymidine incorporation by cortisol (36). However, the effect is maximal when the monocyte content reaches 5%. Our own preliminary data showed no change in the percentage inhibition by various doses of dexamethasone (in the presence of 10 µg/ml ConA) if the monocyte content was reduced from 18 to 3% or if it was increased in stages from 12 to 42%. Therefore, it seems unlikely that the difference in cellular composition biased the comparison between the two groups.

The second possible source of bias in the mitogenesis-inhibition assay lies in the smaller stimulation of [³H]thymidine incorporation by ConA, in the absence of dexamethasone, in the cells of the patients than in the control cells (Fig. 2). The implications of this difference in baseline are uncertain. In PBMLs stimulated by another lectin, phytohaemagglutinin, there is a highly significant positive correlation between the IC_{50} for dexamethasone and [³H]thymidine incorporation in its absence (37), raising the possibility that the greater sensitivity in the injured was secondary to their lower proliferative response to ConA. However, we found no such relationship in either the patients or the control subjects. There was a weak correlation between maximal inhibition and baseline [³H]thymidine incorporation in the patients at a ConA concentration of 1 µg/ml, but not at 10 µg/ml, when the range of values was greater and any important relationship should have become apparent. There is therefore no evidence from our data that the difference in stimulation by ConA was an important source of bias.

What are the implications of the current findings for cortisol actions in hip-fracture patients? The mitogenesis-inhibition assay in PBMLs has been used to investigate peripheral sensitivity to glucocorticoids in many different conditions such as depression (12), asthma (33, 38), rheumatoid arthritis (13) and kidney transplantation (39). Most workers have found it to be of value in identifying patients resistant to treatment with glucocorticoids (13, 33, 39). Such patients do not usually show changes in glucocorticoid-binding parameters in their PBMLs (33, 40). The very rare patients with glucocorticoid resistance due to mutations in GR show reduced ability of glucocorticoids to inhibit mitogenesis as well as impaired binding in PBMLs (41). On this basis it seems reasonable to expect that hip-fracture patients would show normal sensitivity to any immunosuppressive effects of their raised cortisol concentrations.

The question of what such effects might be is an important one, given the raised death rate for several months after proximal femur fracture (4). Increased neutrophil and decreased lymphocyte counts are seen for a short time after injury and have been attributed to the effects of cortisol (42). Animal experiments confirm that the acute neutrophilia and lymphopenia caused by stress are largely mediated by endogenous corticosteroids (43). Since similar changes in white cell counts were seen in our patients (Table 2), it is possible that the persistently raised cortisol concentration was responsible. Whether it is likely to have had effects on the function of these cells is uncertain. Although many of the immunosuppressive effects of trauma can be reproduced by glucocorticoids, the dose-response curves, time courses etc. often argue against a causal relationship (42, 43). It should be noted that the effects of glucocorticoids on the immune system are extremely complex and not all are inhibitory (26, 43). Teleologically such responses may have evolved to prepare the immune system to fight infection (43) or to prevent it from remaining harmfully activated once the infection has been controlled (26). It remains to be seen whether either theory has relevance to hip-fracture patients or whether any immunological effects of their raised cortisol concentrations are merely the result of pathological changes in the hippocampus or elsewhere.

PBMLs are often taken as a paradigm for peripheral tissues in general, and on this basis our results provide evidence against resistance to glucocorticoid effects in non-immunological tissues. However, there is a growing list of possible reasons for variations in glucocorticoid sensitivity between tissues, such as differences in their content of 11β -hydroxysteroid dehydrogenase (44), mineralocorticoid receptors (45), heat-shock proteins (46), other endogenous regulators of GR function (29) and transcription factors mediating other effects (27). Studies of the association between cortisol concentrations and putative glucocorticoid actions in other tissues are now required.

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