Effects of hydrocortisone and adrenaline on natural killer cell activity

Y. NOMOTO, S. KARASAWA and K. UEHARA

Summary
We have studied the effects of hydrocortisone and adrenaline on natural killer (NK) cell activity and on the distribution of circulating lymphocyte subpopulations in 30 patients undergoing elective partial laminectomy under general anaesthesia. The patients were allocated to receive adrenaline (group 1, n = 11), hydrocortisone and adrenaline (group 2, n = 11) or neither hydrocortisone nor adrenaline (group 3, n = 8). Group 1 and group 2 patients received local adrenaline infiltration during operation to reduce bleeding. The mean dose of adrenaline administered was 2.1 (SD 0.2) μg kg⁻¹. Group 2 received hydrocortisone 10 mg kg⁻¹ i.v. after premedication. In groups 1 and 2, adrenaline produced an instantaneous increase in NK cell activity accompanied by a selective increase in circulating NK cells. The measurements returned to pre-infiltration levels within 120 min of administration of adrenaline. The effect of adrenaline in causing increased NK cell activity was not blocked by pre-administration of hydrocortisone. There was a significant decrease in the ratio of T-helper/inducer cells (CD4) to T-suppressor/cytotoxic cells (CD8) in all patients after induction of anaesthesia. In groups 1 and 3, the CD4/CD8 cell ratio did not change significantly during operation. However, compared with groups 1 and 3, group 2 showed a significantly reduced CD4/CD8 cell ratio during operation. Therefore, these results suggest that even in cases of such severe stress that the immune response was depressed by increased serum cortisol concentrations, adrenaline-induced NK cell activity enhancement was preserved. (Br. J. Anaesth. 1994; 73: 318–321)

Key words
Immune response, natural killer cells. Hormones, glucocorticoid. Sympathetic nervous system, adrenaline.

It has been suggested that natural killer (NK) cells play an important role in several host defence mechanisms [1]. Natural killer cell activity fluctuates during anaesthesia and major surgery [2, 3]. The mechanisms underlying these fluctuations in NK cell activity are unknown, but it has been suggested that the stress response is important [4]. Plasma concentrations of adrenaline and noradrenaline and serum cortisol concentration are prominent markers of the stress response and interfere profoundly with NK cell activity. Several studies have shown that adrenaline and cortisol exert opposite effects on the NK cell system. Adrenaline enhances NK cell activity in vitro [5] and in vivo, while cortisol inhibits the activity [6].

Using an i.v. administration pattern to simulate some of the immune changes induced by major surgery and stress, the effects of adrenaline and cortisol on NK cell activity and circulating lymphocyte subpopulations have been investigated.

Patients and methods
With local Ethics Committee approval and informed patient consent, we studied 30 adult patients with lumbar disc herniation or spinal canal stenosis undergoing elective partial laminectomy. The patients were otherwise healthy with normal cardiac, renal and hepatic functions; none had endocrine disorders and none was receiving any medications.

Premedication and general anaesthesia were identical for all patients: premedication comprised atropine 0.5 mg i.m. 1 h before operation; anaesthesia was induced with thiopentone 5 mg kg⁻¹ and tracheal intubation was performed after administration of vecuronium 0.16 mg kg⁻¹. Anaesthesia was maintained by artificial ventilation with enflurane and 66% nitrous oxide in oxygen. In all patients, i.v. infusions during surgery were restricted to Ringer's lactate solution 10 ml kg⁻¹ during the first 1 h and thereafter at 2–4 ml kg⁻¹ h⁻¹.

Thirty patients were then allocated randomly to three groups: group 1 (n = 11) received adrenaline, group 2 (n = 11) received hydrocortisone and adrenaline and group 3 (n = 8) received neither hydrocortisone nor adrenaline. Group 2 patients received hydrocortisone 10 mg kg⁻¹ i.v. after premedication.

Group 1 and group 2 patients received local adrenaline infiltration during operation to reduce bleeding. Adrenaline 1:300000 in normal saline solution was used. The mean does of adrenaline administered was 2.0 (SD 0.2) μg kg⁻¹ in group 1 and 2.1 (0.2) μg kg⁻¹ in group 2. The interval between administration of hydrocortisone and administration of adrenaline was 80 (15) min.

YUKIKO NOMOTO, MD, SYUNJI KARASAWA, MD, KIYOSHI UEHARA, MD, Department of Anaesthesiology, Kanto Rosai Hospital, 2035 Kizukisumiyoshi-cho, Nakahara-ku, Kawasaki-city, Kanagawa-ken 211, Japan. Accepted for publication: March 3, 1994. Correspondence to Y. N.
Hydrocortisone and NK cell activity

Haemorrhage did not exceed 300 ml and no blood substitutes or sympathomimetic agents were required.

Arterial blood samples were obtained, as shown in figure 1, for measurement of NK cell activity, distribution of lymphocyte subpopulations, blood leucocyte counts and measurement of serum cortisol, plasma adrenaline and noradrenaline concentrations. Plasma concentrations of adrenaline and nor-adrenaline were measured by high pressure liquid chromatography. Serum cortisol was measured by radioimmunoassay.

The pan T-cell population (CD3) was measured with Leu4 antibody, the T-helper/inducer cells (CD4) with Leu3a antibody, T-suppressor/cytotoxic cells (CD8) with Leu2a antibody and NK cells (CD16) with Leu11 antibody (Becton Dickinson Monoclonal Center, Mountain View, CA, USA) using flowcytometry (Ortho Spectrum III, Ortho Diagnostic Systems Inc). NK cell activity was measured against K-562 target cells in a chromium-51 release assay. Separation of cells and determination of NK cell activity were performed as described previously [7].

Data are expressed as mean (SD). Statistical analyses were performed using the Wilcoxon test. The level of significance was defined as $P < 0.05$.

Results

There was no difference in age, weight or duration of surgery between the three groups (table 1).

Plasma concentrations of adrenaline reached a peak 30 min after administration of adrenaline in groups 1 and 2. Plasma concentrations of nor-adrenaline did not change significantly during the study. Serum cortisol concentration was 550 (42) μg dl$^{-1}$ 30 min after administration of adrenaline in group 2. In groups 1 and 3, serum cortisol concentrations did not change (fig. 2).

In group 1, adrenaline selectively increased NK cell activity, which reached a peak 30 min after administration of adrenaline and returned to pre-administration values at 120 min. In group 2, an increase in NK cell activity equivalent to the increase caused by administration of adrenaline alone, was demonstrated. There were no significant differences between groups 1 and 2 in NK cell activity or in the number of NK cells. In group 3, NK cell activity did not change significantly during the study (fig. 3).

Compared with preoperative values, CD4 levels decreased after induction of anaesthesia in the three groups. In groups 1 and 3, CD4 levels did not change significantly during operation, after induction of anaesthesia. However, group 2 showed

<table>
<thead>
<tr>
<th>Table 1 Patient data (mean (sd or range))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
</tr>
<tr>
<td>n</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Sex (M/F)</td>
</tr>
<tr>
<td>Age (yr)</td>
</tr>
<tr>
<td>Weight (kg)</td>
</tr>
<tr>
<td>Duration of operation (min)</td>
</tr>
</tbody>
</table>

![Figure 1](image1)

Figure 1 Seven arterial blood samples obtained from each patient before and during operation are indicated by arrows. Sample 1 was obtained after administration of premedication, sample 2 after induction of anaesthesia, sample 3 before administration of adrenaline (A), samples 4, 5, 6 and 7, 15, 30, 60 and 120 min after administration of adrenaline. H = Hydrocortisone.

![Figure 2](image2)

Figure 2 Plasma concentrations of adrenaline (A) and noradrenaline (NA), and serum concentrations of cortisol in group 1 (O) (adrenaline, n = 11), group 2 (●) (hydrocortisone and adrenaline, n = 11) and group 3 (△) (neither hydrocortisone nor adrenaline, n = 8). Sample Nos as in figure 1. $P < 0.05$ compared with: * sample 3; † sample 1.
significantly reduced CD4 levels 120 min after administration of adrenaline. In the three groups, CD8 levels did not change significantly during operation (fig. 4).

In group 1, the CD4/CD8 ratio was 1.8 before induction of anaesthesia and declined to 1.4 before administration of adrenaline. Compared with groups 1 and 3, group 2 showed a significantly reduced CD4/CD8 ratio 60 min and 120 min after administration of adrenaline.

In groups 1 and 2, absolute lymphocytosis occurred but the pan T-cells (CD3) showed no significant change (fig. 4).

In groups 1 and 2, 30 min after administration of adrenaline, mean systolic arterial pressure increased from 120 mm Hg to a peak value of 140 mm Hg, 30 min after administration of adrenaline ($P < 0.05$), but there were no significant changes in diastolic arterial pressure. Heart rate also increased from 78 to 90 beat min$^{-1}$ in both groups.

**Discussion**

We have found that adrenaline selectively increased NK cell activity and increased the absolute number of circulating NK cells *in vivo*. In this study, adrenaline-induced enhancement of NK cell activity was not prevented by pretreatment with hydrocortisone.

The mechanisms of stress-induced changes in immunity are unknown. However, it has been assumed that changes in the immune system response to various types of stress were related to the degree of physiological damage [8]. In cases of severe stress, the immune response was depressed, but in
less severe cases, the immune response was enhanced. Several investigators have suggested that these changes in the immune response were caused by suppressor T lymphocytes being activated by increased concentrations of adrenal cortical hormones in cases of severe stress. On the contrary, helper T lymphocytes activated by increases in the concentration of blood adrenaline released from the adrenal gland were responsible for changes in the immune response in less severe cases. The immune system is also regulated by activation or depression of NK cell in the case of various stresses.

Concentrations of cortisol and adrenaline in this study were comparable with concentrations found during major surgery [2]. By simultaneous administration of adrenaline and cortisol, an approximate simulation of major stress was attempted.

Regulation of NK cell activity is not yet fully understood, but several studies have shown that administration of adrenaline produces an increase in NK cell activity, accompanied by a selective increase in circulating NK cells. The rapid increase in NK cells appears to be explained by mobilization of NK cells from the extravascular space into the circulation. We have demonstrated previously that NK cell activity was also increased by dobutamine [7]. Therefore, adrenaline may cause this mobilization of lymphocytes directly, by activation of lymphoid β receptors, or indirectly by activation of β receptors in other tissues. Stimulation of the NK cell system by adrenaline may thus be part of the general reactions to stress, mediated via the sympatheticadrenal system.

Corticosteroids interfere profoundly with immune responses, in particular therapeutically administered corticosteroids have been found to depress NK cell activity of human peripheral blood [9]. Gatti, Masera and Cavillo investigated the role of Ca$^{2+}$ in mediating inhibition by glucocorticoids of NK cell activity using calcium entry blockers and calmodulin antagonists [10]. They concluded that extracellular and intracellular Ca$^{2+}$ played a role in the control of human NK cell activity.

In our study, the effect of adrenaline in causing increased NK activity was not blocked by pre-administration of hydrocortisone. It was suggested that NK cells were strongly adrenaline-sensitive compared with their sensitivity to hydrocortisone. Therefore, these results suggest that in cases of severe stress when serum cortisol concentration is increased, adrenaline-induced NK cell activity enhancement is preserved.

The human immune system is regulated, in part, by interacting networks of helper/inducer (CD4) and suppressor/cytotoxic (CD8) T cells. The ratio of CD4 to CD8 cells, a commonly used indicator of immunoregulatory cell imbalance, declined after induction of anaesthesia in the three groups in our study. This is in accordance with our previous studies [7].

Although there was no significant effect on the absolute numbers of CD8 cells during operation in group 2, we observed a decrease in CD4 cell numbers and the CD4/CD8 ratio. It has been reported that corticosteroids induced alterations in lymphocyte subpopulations and CD4 cells were more susceptible to the effect of corticosteroids [11]. Corticosteroids decreased the number of CD4 cells, while the number of CD8 cells remained stable. These cortisol-induced changes in the CD4 or CD4/CD8 ratio were similar to the immunosuppressive changes found in cases of severe stress. Therefore, it was suggested that a decrease in the CD4/CD8 ratio was caused, at least in part, by serum cortisol concentration in severe stress. The corticosteroids are used extensively in the treatment of a wide range of inflammatory and immune-mediated diseases. In this study, these cortisol concentrations were comparable with concentrations found during major surgery and were also pharmacological concentrations. We suggest that immunosuppression is a side effect of therapeutically administered corticosteroids, but adrenaline-induced NK cell activity enhancement is not influenced by corticosteroids.

References