Variation in natural killer activity in peripheral blood during the menstrual cycle

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Abstract

Natural killer activity was measured sequentially in normal female volunteers through their menstrual cycle. During the periovulatory period there was a significant fall in natural killer activity compared with in normal male volunteers. This variation was not apparent in women taking oral contraception. Cytotoxic activity was not related to oestradiol concentrations in individual women.

The data support an interaction between immunological activity and sex hormones over the normal physiological range and would account for the described reduction in natural killer activity in pooled blood from female blood donors.

Introduction

Early experiments in animals showed that endocrine manipulation affects immunity.¹ Adrenalectomy and orchidectomy in mice increases the weight of lymph nodes and accelerates the rate of rejection of grafts.² Administration of exogenous hormones also has a pronounced effect on immunity in animals; administration of both oestrogens and androgens affects the number of circulating lymphocytes and the survival of tumour grafts in mice.³ Reports of studies of human cells in culture have claimed that sex hormones modulate the functions of lymphocytes, but results are conflicting.^{4,5} Administration of hormonal preparations may have an effect on immunity in patients with cancer,⁶ and these effects could be important in the host response after treatment against tumours.⁷

The natural killer system in man has been claimed to help in defence against viral and parasitic infections and in resistance to the spread of malignant disease.⁸ In a recent report on breast cancer chance observations related hormonal state to the amount of natural killer activity in the women studied.⁹ The study was not, however, designed to investigate the effect in vivo of sex hormones on cytotoxic activity.

We report the results of an attempt to measure fluctuations in natural killer activity in normal female volunteers through their menstrual cycle. The results are compared with the normal male range.

Subjects and methods

Venous blood was obtained from 18 premenopausal women (mean age 26.6 years) who were not taking oral contraception and 12 women (mean age 24.8 years) who were taking hormonal contraception. The mean age of seven male controls was 28.1 years. Women gave three or four blood samples throughout their menstrual cycle. Venepuncture was performed between 0900 and 1100.

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Mononuclear cells in whole blood were separated out by centrifugation through Ficoll and Triosil and then washed in Hanks's balanced salt solution not containing calcium or magnesium.¹⁰ All reagents for tissue culture were obtained from Gibco Biocult UK. Mononuclear cells were assayed for natural killer activity against the natural killer sensitive cell line K562, as described by White *et al.*⁹ Briefly, 100 μ l of effector cells (4×10^9 /l) was mixed with an equal volume of K562 target cells (1×10^8 /l) previously labelled with 3.7 MBq (100 μ Ci) 51 Cr-sodium chromate (CJS4, Amersham International, United Kingdom). Replicate cultures and controls were incubated at 37°C for four hours in an atmosphere of 5% carbon dioxide in air and the radioactive chromium present in the supernatant determined with a gammacounter. For each set of replicates the specific cytotoxicity relative to cells lysed by detergent was calculated by the formula:

$$\% \text{ specific cytotoxicity} = 100 \times \frac{{}^{51}\text{Cr released by effector cells} - {}^{51}\text{Cr released in medium}}{{}^{51}\text{Cr released by detergent} - {}^{51}\text{Cr released in medium}}$$

To help plot cytotoxicity in relation to the stage in the menstrual cycle and to assess the effect of in vivo hormone concentrations on natural killer activity parallel serum samples from all the women were assayed for oestradiol concentration. Serum samples were

FIG 1—Natural killer activity (●—●) and parallel serum oestradiol concentrations (■—■) throughout menstrual cycle in 18 female volunteers who were not taking oral contraceptives. Mean values in seven male volunteers, tested on four occasions over same experimental period, are also shown (○—○). Values are means and standard deviations; the large standard deviations are characteristic of the natural killer assay system in men and women.²⁵

*Significant variation seen during ovulation (unpaired *t* test, $p=0.008$).

Conversion: SI to traditional units—Oestradiol: 1 pmol/l=0.272 pg/ml.

diluted in carbonate and bicarbonate buffer pH 10.5 and extracted with peroxide free ether. After evaporation of the ether under dry nitrogen at 40°C oestradiol concentrations were determined by radioimmunoassay using Guildhay HPS F21-11A antiserum and tritiated oestradiol tracer (Amersham International). Coefficients of variation between batches for the assay were 7.7% (mean concentration 1870 pmol/l (509 pg/ml), $n=25$), 7.7% (mean concentration 910 pmol/l (248 pg/ml), $n=8$), and 12.4% (mean concentration 570 pmol/l (155 pg/ml), $n=13$).

The percentage of macrophages present in each of the populations of effector cells studied was determined by counting cells that were stained by α -naphthyl acetate esterase that inhibited thioride.¹¹ The

range for cells that were positive for esterase varied in the mononuclear cells studied from 7.5 to 34% but was not correlated with the stage in the menstrual cycle, amount of cytotoxicity, or circulating oestradiol concentration. Viability of cells measured by exclusion with trypan blue remained greater than 95% throughout the study.

Results

Figure 1 presents the pooled natural killer activity in the 18 premenopausal women who were not taking oral contraception, measured sequentially through their menstrual cycle. Determinations were made at a ratio of effector to target cells of 40:1. The circulating oestradiol concentrations measured in parallel serum samples are also shown; these conformed to accepted concentrations and fluctuations of oestradiol during the menstrual cycle. The pooled data showed that the natural killer activity fell during ovulation in the 18 women. The difference in cytotoxicity before and after ovulation was significant (unpaired *t* test, *p* = 0.008).

Figure 2 shows the pooled natural killer activity and parallel serum oestradiol concentrations in the 12 volunteers taking oral contraception. Cytotoxicity in this group did not differ significantly through the cycle.

FIG 2—Natural killer activity (●—●) and parallel serum oestradiol concentrations (■—■) throughout menstrual cycle in 12 female volunteers taking the combined oral contraceptive pill. Mean values in seven male volunteers, tested on four occasions over experimental period, are also given (same control values as in fig 1) (○—○). Values are means and standard deviations; the large standard deviations are characteristic of the natural killer assay system in men and women.²⁵

Conversion: SI to traditional units—Oestradiol: 1 pmol/l = 0.272 pg/ml.

No correlation was seen between the level of cytotoxicity observed in venous blood and the concentration of circulating oestradiol present in a parallel serum sample in individual women, whether or not they were taking oral contraception. The radioimmunoassay used, however, did not measure circulating synthetic oestradiol. Measured concentrations of oestradiol in the women taking oral contraception may not, therefore, have represented the true circulating concentrations.

Discussion

Measurements of natural killer activity in volunteers indicated that women have a lower cytotoxic response than men¹²; other authors have claimed that natural killer activity falls in the third trimester of pregnancy,¹³ though this is disputed.¹⁴ Recently we obtained data that implied that natural killer activity was significantly depressed in women with breast cancer,⁹ an observation also made by other workers.¹⁵ The observed reduction in the function of natural killer cells differed between premenopausal and postmenopausal patients with

the disease, implying an interaction of the function of natural killer effector cells with the endocrine system in women. The study was based on single observations, however, without measurement of hormone concentrations and therefore could not show hormonal effects with confidence.

In this study we investigated the natural killer activity in the peripheral blood in 18 female volunteers at several stages during their menstrual cycle. Parallel estimations of serum oestradiol concentrations conformed well with accepted menstrual fluctuation of this hormone, confirming that cytotoxicity values were correctly placed in the menstrual cycle.¹⁶

The results obtained in the women not taking oral contraception (fig 1) showed a significant depression in natural killer activity around mid-cycle, when ovulation occurs. No such depression was seen in the group taking the combined oral contraceptive pill. Careful studies in the mouse have shown that oestrogen at supraphysiological doses can affect the amount of cytotoxic activity, but there is no evidence of a direct effect on the natural killer effector cell.^{17,18} We were unable to show a correlation between individual natural killer activities and the concentration of oestradiol measured in parallel serum samples. Barrett *et al.*¹⁹ who recently showed that serum obtained during pregnancy inhibited natural killer activity, were similarly unable to relate this effect to the oestrogen concentrations in the blood. The *in vivo* data presented indicate that oestradiol at a physiological concentration does not directly affect the function of natural killer cells. Our inability to measure synthetic oestradiol precludes comment on the effect of oral contraceptives on natural killer activity.

The time around ovulation is characterised by large changes in concentrations of hormones,¹⁶ particularly the polypeptide gonadotrophins, and one or more of these may possibly have mediated the depression of natural killer activity observed in the group of women not taking oral contraceptives. Work in our laboratory has suggested that luteinising hormone at concentrations found *in vivo* in the periovulatory period significantly depresses natural killer activity *in vitro*.²⁰ Human chorionic gonadotrophin, a close analogue of luteinising hormone and known to share the same surface receptor,²¹ similarly depressed natural killer activity *in vitro* at concentrations found in the first and third trimesters of pregnancy. Human chorionic gonadotrophin has other immunomodulatory effects on the responses of lymphocytes *in vitro*,²² and recently natural killer activity was shown to be depressed in pre-eclampsia, when human chorionic gonadotrophin concentrations are raised.²³

In conclusion, this study suggests that fluctuations in the response of the immune system occur during the normal menstrual cycle. The study further suggests that causes of this variation in function of effector cells are more complex than a direct effect of circulating oestrogens and may include interactions with other cyclical hormones, themselves shown to interact with the immune system.²² This type of variation would account for the reduced amount of natural killer activity observed in the women who were not taking oral contraceptives when compared with men²⁴ and may be important in patients undergoing hormone manipulation, particularly for malignancy.

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Somatostatin and the dumping syndrome

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Abstract

Infusion of somatostatin reduced the symptoms of the early dumping syndrome after oral glucose was given and also reduced the associated tachycardia and rise in packed cell volume. It inhibited the secretion of enteroglucagon, neurotensin, and vasoactive intestinal polypeptide, which are raised in patients with the dumping syndrome and may have an aetiological role. It also prevented the reactive hypoglycaemia of late dumping by inhibiting the release of gastric inhibitory polypeptide and insulin.

Somatostatin, possibly through its inhibitory effects on hormonal secretion, may have a role in the management of patients with the early and late dumping syndrome.

Introduction

The dumping syndrome is a complication of gastric surgery and has two phases. Early dumping occurs within 30 minutes after a meal and is thought to be due to the rapid transit of hyperosmolar food into the upper small bowel; this causes secretion of water and electrolytes into the small intestine, haemoconcentration, and thus the characteristic symptoms of sweating, palpitations, faintness, dizziness, abdominal distension, and diarrhoea. In early dumping the plasma concentrations of the gut regulatory peptides, enteroglucagon,¹ neurotensin,² and vasoactive intestinal polypeptide,³ are considerably raised, and it has been suggested that the vasomotor peptides neurotensin and vasoactive intestinal polypeptide may contribute to the

pathogenesis of the symptoms. Late dumping is due to hypoglycaemia several hours after a meal and is attributed to hyperinsulinaemia secondary to the rapid initial rise of blood glucose concentration.

Somatostatin is a peptide made up of 14 amino acids that is found in high concentrations in the pancreas and gut. It inhibits the secretion of numerous hormones in the gut and pancreas, including enteroglucagon, neurotensin, vasoactive intestinal polypeptide, and insulin,^{4,5} and also small intestinal secretion of water and electrolytes in animals⁶ and patients with pancreatic cholera.⁷ These actions suggested that it might be useful in managing the early and late dumping syndrome, and we carried out a study to test this.

Patients and methods

We studied six men with a mean (SEM) age of 50 (5.5). Surgical procedures were two total gastrectomies, two Polya's operations, one gastrojejunostomy and vagotomy, and one vagotomy and pyloroplasty followed by antrectomy and gastrojejunostomy. The last operation had been performed a mean of 11 (3) years previously. During normal life all six patients regularly experienced symptoms of the early dumping syndrome such as faintness, palpitations, sweating, weakness, and somnolence, and one also had the symptoms of reactive hypoglycaemia.

The patients were admitted to hospital and fasted from midnight. The next morning a venous cannula was put into a vein of each arm and basal blood samples were taken at -30, -15, and 0 minutes. At 0 minutes an infusion of isotonic sodium chloride with 0.3 mmol (20 g) human albumin/l was started in the other arm on the control day and somatostatin (Serono Laboratories, UK) 70 pmol (110 ng)/kg/min was added on the treatment day. The control day always preceded the treatment day; the researcher knew what the patient was receiving, but this information was withheld from the patient. After 30 minutes of infusion oral glucose 50 g in 200 ml water was given and the patients assessed for a further three hours. In the three patients who did not develop symptoms of early dumping with 50 g glucose the test was repeated with 100 g glucose in 400 ml water, and dumping was then provoked. The pulse rate was monitored regularly and blood samples collected throughout.

Packed cell volume and serum glucose concentration were monitored by standard techniques. Blood for hormonal radioimmunoassay was collected into heparinised tubes containing 200 µl aprotinin/10 ml blood. The tubes were centrifuged and the plasma decanted within

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