

## Effect of $\beta$ -endorphin and Cortisol on the PHA Stimulated Lymphoblastogenesis

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*The mechanism of immune suppression in a severely stressful condition is not known. Since the demonstration of  $\beta$ -endorphin receptor on the surface of the circulating lymphocyte, it was reported that  $\beta$ -endorphin could suppress PHA stimulated lymphoblastogenesis. Because the concentration of  $\beta$ -endorphin was supraphysiologically high, it is doubtful that  $\beta$ -endorphin can suppress the lymphoblastogenesis directly in vivo. We investigated the suppression of PHA stimulated lymphoblastogenesis by  $\beta$ -endorphin in vitro and the effect of  $\beta$ -endorphin in some conditions where  $\beta$ -endorphin increases in vivo.*

*PHA induced lymphoblastogenesis of T lymphocyte was maximal at the concentration of 5  $\mu$ g/ml.  $\beta$ -endorphin could not suppress the blastogenesis even at the highest concentration. In the five healthy men who received metyrapone the previous night, PHA stimulated blastogeneses were not significantly suppressed. In a patient with Nelson's syndrome, the lymphoblastogenesis was suppressed at all concentrations of PHA.*

*Cortisol significant suppressed the blastogenesis even at the concentration of 10  $\mu$ g/dl and its suppressive effect was shown in dose dependant manner.*

*Our results suggested that  $\beta$ -endorphin could not suppress the lymphoblastogenesis directly in vivo.*

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Key Words:  *$\beta$ -endorphin, Cortisol, Lymphoblastogenesis*

### INTRODUCTION

It has been well known that many stressful conditions, such as trauma,<sup>1)</sup> burn,<sup>2,3)</sup> surgical operations,<sup>1,4,5)</sup> may cause immune suppression which increases the susceptibility to infection. But the mechanism of immune suppression is still not clear.

Occino et al.<sup>6)</sup> demonstrated a polypeptide that is present in human serum, and its molecular weight is below 10,000 dalton. Menzoian et al.<sup>7,8)</sup> suggested that the polypeptide suppresses many immunologic reactions which are mediated by T lymphocyte. Also, Viet and Mechael demonstrated an inhibitor

of immunologic response increased whenever the immune system was stimulated.  $\beta$ -endorphin, m.w. 3450, is released with ACTH from the pituitary gland under stressful conditions<sup>10,11,12)</sup> and its receptor has been demonstrated on the circulating lymphocytes.<sup>13)</sup> McCain et al.<sup>14)</sup> demonstrated that PHA stimulated lymphoblastogenesis could be suppressed by the high concentration (10<sup>5</sup> pM) of  $\beta$ -endorphin. Because their concentration of  $\beta$ -endorphin was also much higher than that (15-30 pM) of the patient who received stress and it was doubtful that the defect of lymphoblastogenesis caused the suppression of immunologic response, we observed the suppression of PHA stimulated lymphoblastogenesis in two conditions, metyrapone test and Nelson's syndrome, in which plasma  $\beta$ -endorphin concentration increases. Simultaneously, we observed the effect of cortisol on the PHA

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stimulated lymphoblastogenesis because the hormone increase in the stressful conditions.

### SUBJECTS AND METHODS

Between 9 a.m. and 10 a.m. 20 ml of whole blood was withdrawn from the antecubital vein and was mixed with one volume of Eagle's minimal essential media. Mononuclear cells were isolated from the heparinized blood over a Ficoll-Hypaque gradient and washed two times with Hank's balanced salt solution. The mononuclear cells were suspended in complete media (minimal essential media including 10% fetal bovine serum, streptomycin and amphotericin B) to make the concentration  $2 \times 10^5$ /ml.

Phytohemagglutinin (PHA) (Lectin, Sigma Co.) was diluted to the concentration 0,1,2,5,7,10  $\mu$ g/ml with complete media.  $\beta$ -endorphin (Sigma Co.) was diluted to make final concentration 10,10<sup>2</sup>,10<sup>4</sup>

pM/well and cortisol (Sigma Co.) was also diluted to the final concentration 10,10<sup>2</sup>, 10<sup>3</sup>  $\mu$ g/dl. 100  $\mu$ l of cell suspension was incubated with 100  $\mu$ l of PHA and 20  $\mu$ l of  $\beta$ -endorphin or cortisol in the round bottomed microtitration plates for 48 hours under 5% CO<sub>2</sub>,37°C. After 6 days of culture, 10  $\mu$ l of 0.1  $\mu$ Ci of <sup>3</sup>H-thymidine (specific activity 2Ci/mmol, New England Nuclear) was added to the wells and incubated for 20 hours. Cells were harvested and dried at 37°C for 60 min. before counting with liquid scintillation spectrophotometer. The viability of cells were determined by the trypan blue dye exclusion method. Suppression of lymphoblastogenesis was expressed with % suppression.

Metyrapone (1.5 gm) was administered to five healthy men at 10 p.m. and whole blood was withdrawn at 9 a.m. in the next morning. The PHA stimulated lymphoblastogenesis was done in a patient with Nelson's syndrome.

### RESULTS

The maximal lymphoblastogenesis was observed at the PHA concentration 5  $\mu$ g/ml.  $\beta$ -endorphin did not suppress the lymphoblastogenesis at all concentrations (Fig. 1, 2).

In the cases who received metyrapone the previous night, the lymphoblastogenesis was suppressed 27 percent in the wells containing no PHA (Fig. 3, 4) although there was no suppression in the wells containing PHA.

In the patient with Nelson's syndrome, T lymphoblastogenesis were suppressed 60%, 30%,

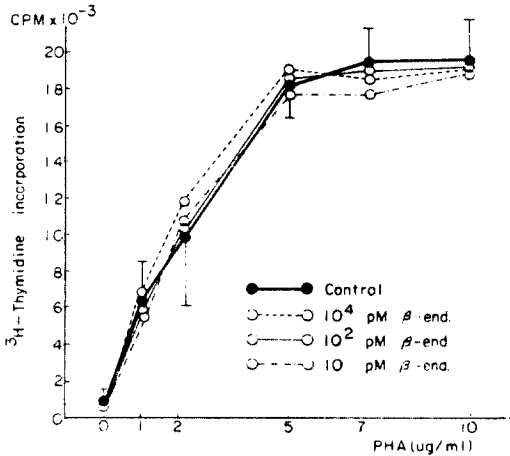


Fig. 1. The effect of  $\beta$ -endorphin on PHA stimulated blastogenesis of normal human T lymphocytes.

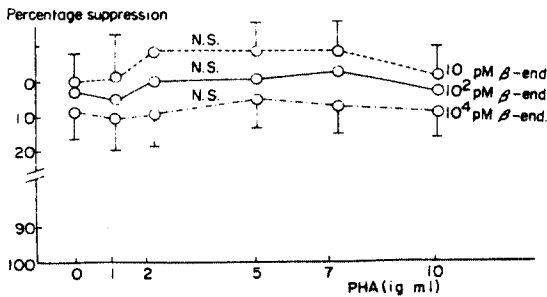


Fig. 2. The percentage suppression of PHA stimulated blastogenesis of normal human T lymphocytes by  $\beta$ -endorphin.

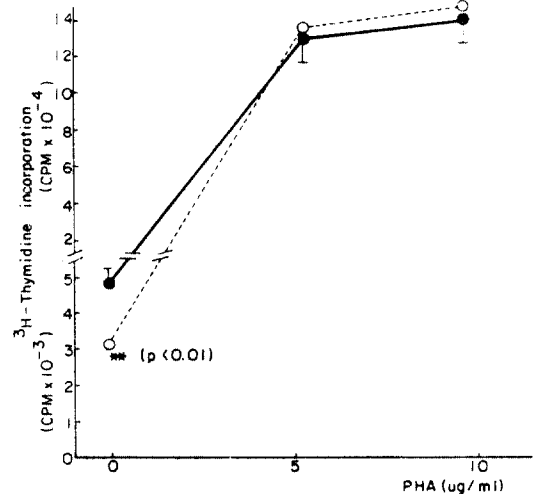


Fig. 3. The PHA stimulated blastogenesis of T lymphocytes in normal subjects stimulated with metyrapone.

25% at each concentration (0, 5, 10 ug/ml) of PHA (Fig. 5, 6).

Cortisol suppressed the T lymphoblastogenesis in a dose dependent manner and the maximal suppressive effect was seen at the PHA concentration of 1 ug/ml (Fig. 7, 8).

DISCUSSION

Severe stress may influence the immune system, resulting in the development of a disease.<sup>1-5, 15-18</sup>

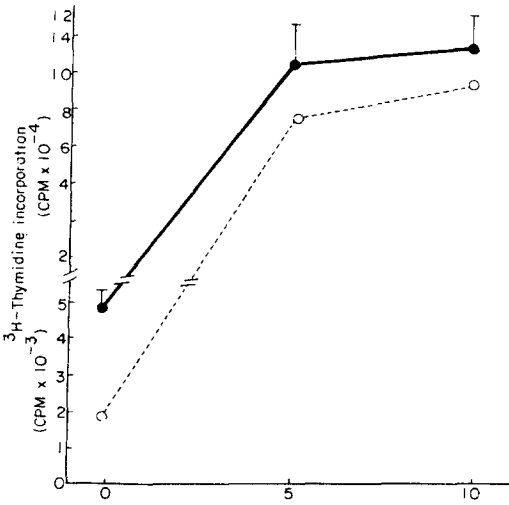


Fig. 5. The suppression of PHA stimulated blastogenesis of T lymphocytes in a patient with Nelson's syndrome.

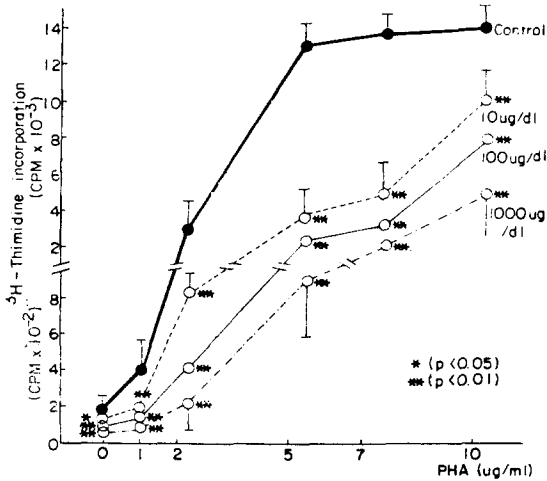


Fig. 7. The suppressive effect of cortisol on the PHA stimulated blastogenesis of T lymphocytes in normal subjects.

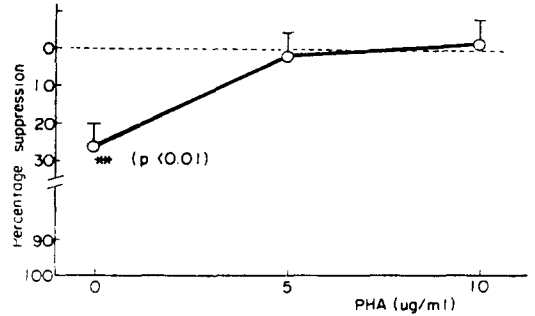


Fig. 4. The percentage suppression of the PHA stimulated blastogenesis of T lymphocytes in normal subjects stimulated with metyrapone.

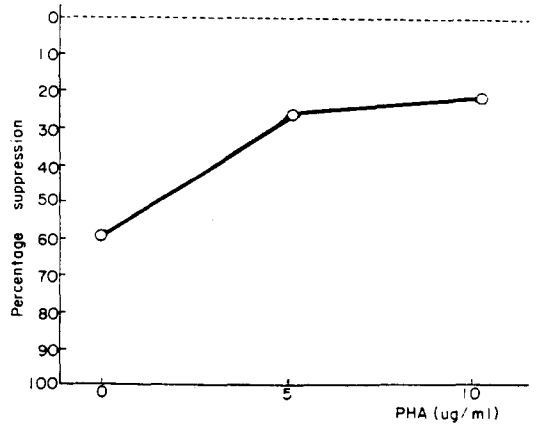


Fig. 6. The percentage suppression of the PHA stimulated blastogenesis of T lymphocytes in a patient with Nelson's syndrome.

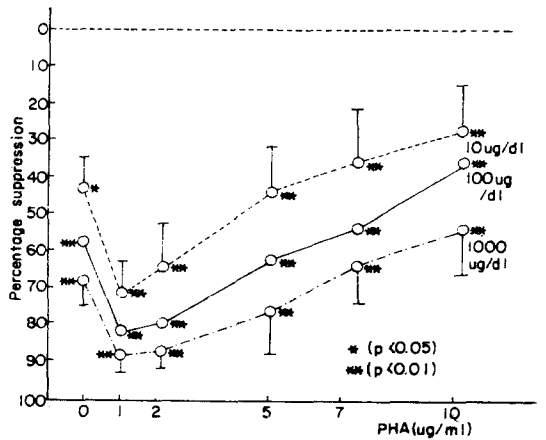


Fig. 8. The percentage suppression of the PHA stimulated blastogenesis of T lymphocytes by cortisol in normal subjects.

But the mechanism by which the stress can alter the immune function remains unclear.

During the past decades there has been many efforts to find which substance has a capacity to alter the immune function. In 1959, Karmrin<sup>19)</sup> isolated a protein fraction from human plasma that could suppress the immune function, and Mowbray et al.<sup>20)</sup> successfully suppressed the rejection and antibody formation by using a  $\alpha$ -globulin fraction isolated from the bovine serum. Occhino et al.<sup>6)</sup> isolated a peptide subfraction, m.w. 4000 to 9000, from the  $\alpha$ -globulin fraction and also Constantian et al.<sup>11)</sup> isolated a peptide, m.w. below 10,000, from the patients who received trauma or surgery which suppressed the PHA stimulated lymphoblastogenesis. Although it has not been known what it is, it seems a possible hypothesis that a hormone can modulate the immune system under stressful conditions because the endocrine system responds rapidly to the various stress.

$\beta$ -endorphin is a peptide hormone, m.w. 3450, released with ACTH from the pituitary gland in response to stress. It was demonstrated that the peptide hormone has a receptor on the surface of the circulating lymphocytes<sup>13)</sup> and so it was speculated that  $\beta$ -endorphin may modulate immunologic functions. McCain et al.<sup>14)</sup> observed that  $\beta$ -endorphin could suppress the PHA (10ug/well) stimulated lymphoblastogenesis, 30% suppression at  $10^{19}$ M and 75% suppression at  $10^{-7}$ M, and which suggested that  $\beta$ -endorphin act through non-opiate receptor because those suppressive effects could not be inhibited by naloxone. Although we could not observe the suppressive effect of  $\beta$ -endorphin at any concentrations of PHA, our result was not in contrast to their result because our PHA concentrations were different from theirs. Their concentrations were ten times as high as ours because their concentration unit ug/well as compared to ours ug/ml. The suppressive effect was not observed at the low concentration as ours.

But it is thought that  $\beta$ -endorphin has no suppressive effect on PHA stimulated lymphoblastogenesis. There are several reasons as followings: First, even though the PHA concentrations were much lower than theirs, the maximal lymphoblastogenesis was observed at 5 ug/ml (0.5 ug/well) in our experiment. Therefore, the effect should have been observed in our experiment if  $\beta$ -endorphin had a suppressive effect. Second, Goodwin et al.<sup>21)</sup> demonstrated that the suppressive effects of various suppressive substances were observed better at the lower PHA concentration than

the concentration which stimulates maximal lymphoblastogenesis because the higher concentration induces unphysiological conditions. On that point of view, McCain's results were contradictory: the maximal suppressive effect was observed at a higher PHA concentration and minimal suppression at a lower PHA concentration. It seems likely that their results could not implicate the physiological meaning even if suppression was observed. Third, concentrations of  $\beta$ -endorphin used in their experiment were 1,000 times as high as those in the plasma of one who is under a stressful condition, and which also suggest that  $\beta$ -endorphin could not change the lymphoblastogenesis in vivo.

In order to observe the suppressive effect of  $\beta$ -endorphin indirectly in vivo, we investigated the lymphoblastogenesis in 5 healthy men to whom metyrapone was administered and in a patient with Nelson' syndrome. Although their lymphoblastogenesis were not significantly different from those of normal controls when stimulated with PHA, those were suppressed without PHA stimulation. It was thought that the suppressive effect of  $\beta$ -endorphin decreases as the PHA concentration increases. Although it can not be mentioned in general, chronic exposure upon the high concentration of  $\beta$ -endorphin might suppress lymphoblastogenesis in vivo. The difference between in vitro results and in vivo results can not be explained now, not only because an effect of ACTH and an effect caused by cortisol deficiency are not known, but because it is possible that a intermediate step exist in vivo between the elevated  $\beta$ -endorphin and suppression of lymphoblastogenesis.

Blalock et al.<sup>22,23)</sup> demonstrated that leukocyte interferon is a peptide which has similar structures as ACTH and  $\beta$ -endorphin and can be cleaved by the proteolysis to yield ACTH and  $\beta$ -endorphin. It can be hypothesized that the ACTH and  $\beta$ -endorphin cleaved from interferon have a negative feedback effect on the hypothalamus and pituitary gland to suppress the release of ACTH and  $\beta$ -endorphin, and which suggests a close connection between the endocrine system and immune system. Upon the infection, leucocytes synthesize the interferon which suppress the lymphoblastogenesis and simultaneously suppress ACTH and  $\beta$ -endorphin to reduce cortisol secretion. On the other hand, the stress increases the  $\beta$ -endorphin secretion which stimulate synthesis of interferon in lymphocytes as a defence mechanism against the infection, but under prolonged stress, interferon has a negative feedback to decrease a release of ACTH and  $\beta$ -endorphin

resulting in a decrease of interferon synthesis. In the cases of a metyrapone test and a patient with Nelson's syndrome, it can be speculated that lymphoblastogenesis is suppressed by the mechanism of the above hypothesis and a patient with Nelson's syndrome is susceptible to infections.

It is well known that cortisol suppresses the lymphoblastogenesis *in vitro*.<sup>24-28)</sup> In our results, cortisol suppressed the PHA stimulated lymphoblastogenesis in a dose dependent manner. It agrees with other reports and suggests that our experimental method used in the evaluation of suppressive effect of  $\beta$ -endorphin was not wrong. The suppressive effect of cortisol *in vitro* has been explained by the direct toxic effect to lymphoblast<sup>24)</sup> but cortisol has no direct toxic effect even at the pharmacological concentration *in vivo*. Yu et al.<sup>29)</sup> and Fauci et al.<sup>30)</sup> suggested that the circulating lymphocytes decreased, especially T lymphocytes, when cortisol concentration increased resulting in the suppressed PHA stimulated lymphoblastogenesis. Tavida et al.<sup>31)</sup> demonstrated the negative correlation between the serum cortisol concentration and PHA stimulated the lymphoblastogenesis and suggested that suppression of lymphoblastogenesis is not controlled directly by cortisol but by other factors. The above hypothesis can explain the negative correlation; Decrease of cortisol concentration induces  $\beta$ -endorphin secretion which stimulates synthesis of lymphocyte interferon via its receptor, resulting in suppression of lymphoblastogenesis.

In conclusion, we suggested that  $\beta$ -endorphin could not suppress the PHA stimulated lymphoblastogenesis directly *in vitro* in contrast to cortisol, but we speculated that  $\beta$ -endorphin can suppress the lymphoblastogenesis by the stimulation of interferon synthesis indirectly *in vivo*. In the future the studies regarding the relationship between  $\beta$ -endorphin and interferon activity are needed.

## REFERENCES

- Constantian MB, Menzoian JO, Nimberg RB, Schmid K, Mannick JA: Association of a circulating immunosuppressive polypeptide with operative and accidental trauma. *Ann Surg* 18 5:73, 1979
- Alexander JW: Effect of thermal injury upon the early resistance to infection. *J Surg Res* 8:1882, 196
- Farrell MF, Dry NK, Tskradlader V: Study of lymphocyte depletion and serum complement perturbation following acute burn trauma. *Surgery* 73:697, 1973
- Slade MS, Simmons RL, Yunis E, Greenberg LJ: Immunodepression after major surgery in normal patients. *Surgery* 78:363, 1975
- Goodman JS, Schaffner W, Collins, Batters by EJ, Koenig MG: Infection after cardiovascular surgery. *N Engl J Med* 278:117, 1968
- Occhino JC, Glasgow AH, Cooperband SR, Mannick JA, Schmid K: Isolation of an immunosuppressive peptide fraction from human plasma. *J Immunol* 110:685, 1973
- Menzoian JO, Glasgow AH, Nimberg RD, Cooperband SR, Schmid K, Saporoschety, I, Mannick JA: Regulation of T lymphocyte function by immunoregulatory. *J Immunol* 113:266, 1974
- Menzoian JO, Glasgow AH, Nimberg RB, Constantian MB, Stevens RL, Cooperband SR, Schmid K, Mannick JA: The prolongation of renal allograft survival by an immunosuppressive peptide isolated from human plasma. *Transplantation* 18:391, 1974
- Viet BC, Michael JG: Immune-response suppression by an inhibitor in normal and immune mouse serum. *Nature* 235:238, 1972
- Guillemin R, Vargo T, Rossier J, Manick S, Ling N, River C, Vale W, Bloom FE:  $\beta$ -endorphin and adrenocorticotropin are secreted concomitantly by the pituitary gland. *Science* 19:1367, 1977
- Rossier J, French ED, River C, Ling N, Guillemin R, Bloom FE: Foot-shock induced stress increases  $\beta$ -endorphin levels in blood but not brain. *Nature* 270:618, 1977
- Kruger DT, Liotta AS, Suda T, Yamaguchi H: Plasma ACTH, lipotropin and endorphin in human. *Brain and pituitary peptides. Ferring Symp* p. 35, 1979
- Hazum E, Chang KJ, Cuatrecasas P: Specific nonopiate receptors for  $\beta$ -endorphin. *Science* 205:1033, 1979
- McCain HW, Lamster IB, Bozzone JM, Grabic JT:  $\beta$ -endorphin modulates human immune activity via non-opiate receptor mechanisms. *Life Science* 31:1619, 1982
- Keller SE, Schleifer SJ, Miller NE, Stein M: Suppression of immunity by stress: Effect of a graded series of stressors on lymphocyte stimulation in rat. *Science* 213:1397, 1981
- Monjan AA, Collector MI: Stress-induced modulation of immune response.
- Bartrop RW, Lararus L, Lucknurst E, Kiloh LG: Depressed lymphocyte function after bereavement, *Lancet* 1:834, 1977
- Eskola J, Runskanon O, Soppi F, Viljanen NK, Toivonen M, Kouvalainen K: Effect of sport stress on lymphocyte transformation and antibody formation. *Clin Exp Immunol* 32:329, 1978
- Kamrin BB: Successful skin homografts in mature non-

- littermate rats treated with fractions containing alpha-globulins. Proc Soc Exp Biol Med* 100:58, 1959
20. Mowbray JF: *Ability of large doses of an alpha plasma protein fraction to inhibit antibody production. Immunology* 6:217, 1963
  21. Goodwin JS, Messner RP, Williams RC Jr: *Inhibitors of T-cell mitogenesis: Effect of mitogen dose. Cellular immunol* 45:303, 1979
  22. Blalock JE, Smith EM: *Human leukocyte interferon; Structural and biological relatedness to adrenocorticotrophic hormone and endorphins. Proc Natl Acad Sci USA* 77:5972, 1980
  23. Blalock JE, Smith EM: *Human leukocyte interferon (HuLFN-2): Potent endorphin-like opioid activity. Bioch Biophys Res Comm* 101:472, 1981
  24. Heilman DH, Gambrell MR, Leichner JP: *The effect of hydrocortisone on the incorporation of tritiated thymidine by human blood lymphocyte cultured with phytohemagglutinin and pokeweed mitogen. Clin Exp Immunol* 15:203, 1973
  25. Tormey DC, Fudenberg HH, Kamin RM: *Effect of prednisolone on synthesis of DNA and RNA by human lymphocytes in vitro. Nature* 213:281, 1967
  26. Mendelsohn J, Multer MM, Bernheim JL: *Inhibition of human lymphocyte stimulation by steroid hormones: Cytokinetic mechanisms. Clin Exp Immunol* 27:127, 1977
  27. Wyle FA, Kent JR: *Immunosuppression by sex steroid hormones I: The effect upon PHA-and PPD-stimulated lymphocytes. Clin Exp Immunol* 27:407, 1977
  28. Ogawa K, Sueda K, Matsui N: *The effect of cortisol, progesterone, and transcortin on phytohemagglutinin-stimulated human blood mononuclear cells and thier interplay. J Clin Endocrinol Metab* 56:121, 1983
  29. Yu, DTY, Clements PJ, Paulus HE, Peter JB, Levy J, Barnett EV: *Human lymphocyte subpopulations. J Clin Invest* 53:565, 1974
  30. Fauci AS, Dale DC: *The effect of in vivo hydrocortisone on subpopulations of human lymphocytes. J Clin Invest* 53:240, 1974
  31. Tavadia HB, Fleming KA, Hume PD, Simpson HW: *Circadian rhythmicity of human plasma cortisol and PHA-induced lymphocyte transformation. Clin Exp Immunol* 22:190, 1975
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