Acta Biologica Hungarica 54 (3–4), pp. 335–346 (2003)

EFFECT OF ADRENALECTOMY ON RAT PERITONEAL MACROPHAGE RESPONSE

B. BISHAYI,[1]* S. GHOSH[2] and P. BHANJA[3]

[1] Immunology Laboratory, Department of Physiology, University of Calcutta, 92 A.P.C. Road, Calcutta-700 009, India
[2] Immunology Laboratory, Department of Physiology, University of Calcutta, India
[3] Immunology and Microbiology Special Paper, Department of Physiology, University of Calcutta, India

(Received: November 20, 2001; accepted: October 25, 2002)

Glucocorticoid hormones are important for vital functions and act to modulate inflammatory and immune responses. In contrast to other hormonal systems no endogenous mediators have been identified that can directly counter-regulate their potent anti-inflammatory and immunosuppressive properties. Glucocorticoids are known to interfere with the ability of the macrophage not only to induce and amplify an immune response but also to inhibit macrophage inflammatory effector functions. Although the actual immunocompetence of animals undergoing endocrine gland ectomy has never been directly studied, there is no doubt that adrenal hormones are deeply involved in the development and maintenance of the immunitory functions and this may in turn influence the inflammatory reaction.

To study the effect of endogenous glucocorticoids on the functions of rat peritoneal macrophages and induction of humoral immune response we observed some of the rat peritoneal macrophage effector functions, provided that endogenous glucocorticoids are depleted by adrenalectomy. The mean phagocytic index (PI) of control macrophage (Mϕ) is increased from 23,825 ± 427 to 31,895 ± 83 after adrenalectomy ($P \leq 0.001$). Intracellular killing capacity in control cell is 82% which is found to be 73% in case of adrenalectomised cell ($p < 0.05$). The amount of nitric oxide released from control Mϕ 20.25 ± 1 μM following adrenalectomy shows the amount of nitric oxide release was 18.25 μM ($p \leq 0.01$). The percentage of DNA fragmentation in control Mϕ was 68.82 ± 4 which was reduced to 56.76 ± 1 after adrenalectomy ($p \leq 0.01$). In sheep red blood cell (SRBC) immunised and adrenalectomised animal, agglutination titre was obtained at lowest antibody concentration (1 : 128) whereas serum from SRBC immunised normal rats showed early agglutination (1 : 32). Endogenous glucocorticoid depleted rats show enhanced phagocytic capacity, antibody raising capacity as well as on the other hand adrenal hormone insufficiency reduces the intracellular killing capacity, nitric oxide (NO) release, improper cell maturation and heightens the probability of infection. These observations demonstrate a counter-regulatory system via glucocorticoid that functions to control inflammatory and immune responses.

Keywords: Glucocorticoids – adrenalectomy – immunomodulation – inflammation – rat macrophage functions

* Corresponding author; e-mail: biswadev@cucc.ernet.in

INTRODUCTION

The importance of corticosteroid in regulating virtually every component of the immune and inflammatory response including thymocyte growth and differentiation, has long been appreciated [10, 30]. Failure to produce adequate amounts of corticosteroids may promote the development of autoimmune disease [14]. Apoptosis or programmed cell death of rodent thymic lymphocytes has long been recognized following exogenous pharmacological corticosteroid administration [16]. The apoptotic death of thymocyte in the setting of stress was dependent upon endogenous corticosteroids because it was blocked by RU-486, a corticosteroid receptor antagonist [9] also supporting that adult adrenalectomy lead to marked enlargement of the thymus. Corticosteroid have been used to suppress pathological immune responses associated with auto-immunity, inhibit rejection of allogenic tissues after organ transplantation and to diminish inflammation associated with a wide variety of hypersensitivity reactions [1]. At the molecular level neuro-immune mediator molecules or their receptors may be members of the same super family or may regulate each other's expression or function [31]. Immunomodulatory effect of glucocorticoids has been demonstrated [7, 24]. However the exact mechanism of their influence on the immune system remains unclear. Whether hormonal insufficiency (e. g. after adrenalectomy) leads to any modulation in the function of immunocompetent cells during the peak of immune responsiveness is not clear and needs investigations in details. Although the actual immunocompetence of animals undergoing endocrine gland ectomy has never been directly studied, there is no doubt that adrenal hormones are deeply involved in the development and maintenance of immunitory functions and this may in turn influence the inflammatory reaction. Depletion of endogenous glucocorticoid (GC) by adrenalectomy or by injection of saturating amounts of GC receptor antagonist RU-38486 (mife pristone) was found to cause clonal deletion of peripheral T cells *in vivo* [8, 19]. Elevated prostaglandin production, cyclooxygenase (COX) enzyme synthesis and activity were also observed in murine peritoneal macrophages of adrenalectomised animals [6, 13]. Inhibition of thymus glucocorticoid biosynthesis was found to increase in thymocyte apoptosis and a decrease in recovery that was directly proportional to the number of Major Histocompatibility Complex (MHC) encoded molecules present [28, 29]. The most convincing studies of GC with doses comparable to therapeutic ones concerned killing of subpopulation of mouse thymocytes *in vivo* [3]. Several recent reviews have examined glucocorticoid–lymphocytes interaction [4, 20]. Virtually all endogenous glucocorticoids are presumably derived from the adrenal glands and are not locally produced. Glucocorticoids synthesized in the cortex of adrenal gland helps to regulate inflammatory response [21]. The levels of glucocorticoid that modulate lymphocyte function are attainable physiologically specially under condition of stress [2]. Whether adrenalectomised animal is immunologically more potent (since glucocorticoid induced immunosuppressive effect is withdrawn) or not is a pertinent question. Whether there is any change in the functional properties of immunocompetent cells due to adrenalectomy is also an obvious question. Here attempts have been made to

study the functions of rat peritoneal macrophages from adrenalectomised animals and to determine the cause of their fuctional alteration which may lead to immunomodulation. In the present study we have reported that in hormonal insufficiency (due to adrenalectomy) the phagocytic and antibody raising capacity of peritoneal macrophage increases, whereas the intracellular killing capacity, nitric oxide (NO) release also decreases suggesting a possible role of GC in immunomodulation.

MATERIALS AND METHODS

Materials

Male Swiss Albino rats of average body weight 110 g were purchased from local suppliers to our department. Bovine Serum Albumin (BSA), Hydroxy Ethyl Piperazine Ethane Sulfonic Acid (HEPES), Agarose, Giemsa were purchased from Sigma Chemical Co., St. Louis, USA. RPMI-1640 was purchased from Gibco. *S. aureus* MC524 strain was maintained in our laboratory. All other reagents and chemicals were of analytical grade and were divided into two groups, control and adrenalectomised comprising of six animals per group.

Methods

Adrenalectomy

Male Swiss Albino rats of average body weight 110 g were used for adrenalectomy. Surgical removal of the gland under ether anaesthesis was done for adrenalectomy with slight modification as described elsewhere [26]. Control animals were sham operated. After adrenalectomy the animals were administered glucose solution and were housed in plastic cages in departmental animal house provided with sufficient food and water.

Separation of rat peritoneal macrophages

5% (w/v) bacto-peptone in saline (5 ml/100 g body weight) was injected intraperitoneally (i.p.) into rats and peritoneal exudate cells were collected on day 4 by washing the cavity with 20 ml of ice-cold Ca^{2+} free Hank's balanced salt solution (HBSS). Cells were washed twice and plated onto 60 mm plastic dishes in RPMI-1640 containing 1% (w/v) BSA. After 2 h incubation at 37 °C, non adherent cells were removed by rinsing. The RPMI-1640 medium containing 1% BSA was added to the adhered cells and they were used as macrophages [15]. The viability of macrophages was >95% as assessed by Trypan blue dye exclusion.

Phagocytosis assay

Cells in HBS-BSA (100 µl from 10^6/ml) were allowed to adhere onto glass slides for 1 h. Then 100 µl of 10% shep red blood cells (SRBC) was added onto the glass slides on which macrophages (Mϕs) have been adhered previously. The slides were incubated for 3 h at 37 °C. Then the cells were washed in HBSS, fixed in 50% methanol and stained with Giemsa, and observed under microscope. Phagocytic index (PI) was calculated as described earlier [5]:

$$\text{Phagocytic index} = \text{Percentage of M}\phi \text{ that contains SRBC} \times \text{Average no. SRBC per M}\phi\text{s} \times 100$$

Intracellular killing

Equal volume of 1×10^7 macrophages/ml and bacteria in HBSS-gelatin were incubated at 37 °C under slow rotation for 3 min. Phagocytosis was stopped by shaking the tubes in crushed ice and free bacteria were removed by centrifugation and washing. Then cells containing ingested bacteria were reincubated at 37 °C for different times. At different time intervals, cells were taken and intracellular killing was terminated by keeping the cells in ice, followed by centrifugation. After addition of distilled water containing 0.01% BSA to the pellet, cells were disrupted by vigorously vortexing which causes release to intracellular bacteria in the supernatant. Then the viable intracellular bacteria in the supernatant were determined microbiologically by colony counting method. The number of colonies obtained at the beginning of the experiment (zero minute after reincubation) was designated as given by 100% viable bacteria. Intracellular killing was then expressed as the percentage decrease from the initial number of viable bacteria [12, 25].

Nitric oxide release

Cells were suspended in Dulbecco's Phosphate Buffered Saline (DPBS) – BSA and were stimulated with Lipopolysaccharide (100 ng/ml) for 30 min at 37 °C, after centrifugation at 10,000 rpm for 15 min. Cell free supernatant was collected in separate micro-centrifuge tube and was used for nitric oxide release assay. Each of the cell free supernatants (200 µl) was reacted with Griess reagent (200 µl) containing 1 part of 1% sulfanilamide in 5% phosphoric acid and 1 part of 0.1% (1 naphthyl) – ethylene diamine dihydrochloride was added and incubated for 10 min at room temperature. Readings were taken in a spectrophotometer at 550 nm compared to a sodium nitrite ($NaNO_2$) standard curve (ranging 0.5–25 µM) and the amount of NO released in the supernatant was measured in each set [23].

DNA fragmentation assay

Cells (10^6 cells/200 µl) were resuspended in hypotonic lysis buffer [0.2% Triton X-100, 10 mM Tris, 1 mM Ethylene Diamine Tetra Acetic Acid (EDTA), pH = 8.0] and centrifuged for 15 min at 14,000×g. The supernatant containing small DNA fragments was collected and the cell pellet containing large pieces of DNA and cell debris was used for diphenylamine (DPA) assay. The DPA reaction was performed by the method of Ochiai et al. [18].

Perchloric acid (0.5 M) was added to the pellets containing uncut DNA (resuspended in 200 µl hypotonic lysis buffer) and to the other half of the supernatant containing DNA fragments and then 2 volumes of a solution containing 0.088 M diphenylamine (DPA), 98% (v/v) glacial acetic acid, 1.5% (v/v) sulfuric acid and 0.5% (v/v) of 1.6% acetaldehyde solution were added. The samples were stored at 4 °C for 48 h. The colorimetric reaction was quantitated spectrophotometrically at 575 nm using an UV spectrophotometer:

$$\text{Percentage of DNA Fragmentation} = \frac{\text{Reading of Supernatant}}{\text{Reading of Pellet} + \text{Reading of Supernatant}} \times 100$$

Immunoagglutination for detection of humoral immune response

Freshly prepared 10% SRBC was used as particulate antigen for immunisation. 100 ml of this solution was injected intraperitoneally per rat on the second day after adrenalectomy. Intraperitonial (i.p.) injection of SRBC was continued for two weeks at a schedule of 3 doses per week with an interval of one day between the consecutive doses. Blood was collected from the retro-orbital plexus of both control and adrenalectomised rats and serum was prepared after clot retraction. Serum of both groups was serially diluted twofold in a series of tubes to which 10% SRBC was added. The last tube showing visible hemagglutination will reflect the serum antibody titre [11].

RESULTS

Phagocytic capacity of rat peritoneal macrophages in glucocorticoid (GC) insufficiency after adrenalectomy

In order to address whether there was any alteration in the phagocytic capacity of rat peritoneal macrophages in hormonal insufficiency due to adrenalectomy, phagocytosis of peritoneal Mϕs against SRBC was assayed. It was observed that the phagocytic index (mean ± S.D.) of control cell was 23,825 ± 427, which was significantly enhanced in cell of adrenalectomised animals 31,895 ± 83 ($p \leq 0.001$) (Fig. 1).

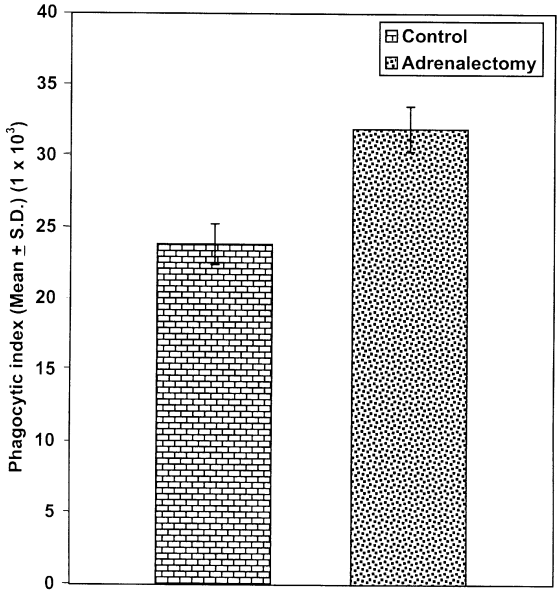

Fig. 1. Effect of adrenalectomy on the phagocytic capacity of rat peritoneal macrophages. Values are mean P.I. of three independent experiments ±S. D. ($p \leq 0.001$)

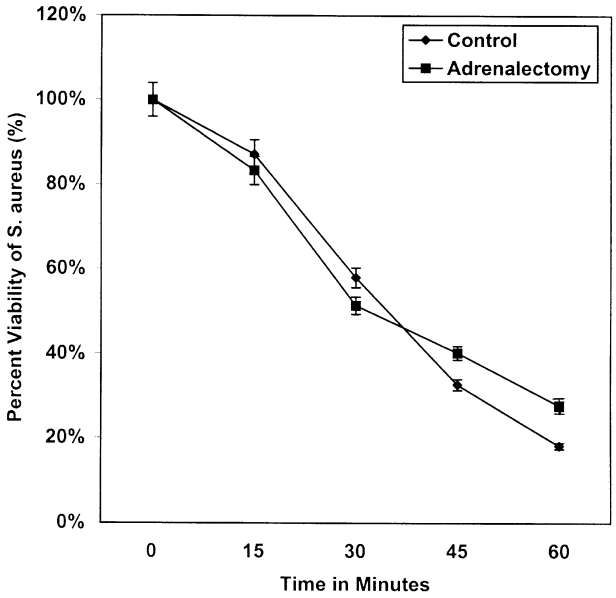

Fig. 2. Intracellular killing capacity of endogenously glucocorticoid depleted rat peritoneal macrophages. Intracellular killing was expressed as the percentage decrease in initial number of viable intracellular bacteria. The result in this figure represents the decrease (%) in viability of *S. aureus* from different groups of three independent experiments ($p < 0.05$)

Intracellular killing capacity of rat peritoneal macrophages in GC insufficiency after adrenalectomy

During phagocytosis specialised cells such as peritoneal Mϕs take up and degrade bacteria that need to be eliminated from the body. Whether adrenalectomy can lead to any change in the aforesaid function of Mϕs was determined. The results showed that intracellular killing capacity was maximum at 60 min in case of control, since they have only 18% viable bacteria in their extracellular milieu or rather they kill 82% bacteria, whereas, in case of cells recovered from adrenalectomised animals at 60 min they have 27% viable bacteria i.e., they can kill near about 73% bacteria which was less than that of control group. It was also observed that the viability of bacteria gradually decreases in control cells with time, whereas in cells of adrenalectomised animal, the viability of *S. aureus* gradually increases (at 45 and 60 min) which indicates that peritoneal Mϕs during adrenal hormonal insufficiency cannot ingest *S. aureus* properly, so more bacteria were left viable by them ($p < 0.05$) (Fig. 2).

Nitric oxide release from rat peritoneal macrophages in GC insufficiency after adrenalectomy

Nitric Oxide (NO) itself has potent anti-microbial activity and it can also combine with superoxide anion to yield even more potent anti-microbial substances. Therefore, a modulation in the release of NO could designate a decline in the animal's ability to defend itself from microbes. The effect of adrenalectomy on the NO release seeks to demonstrate the immunomodulatory effect of endogenous hormones. It was observed from the spectrophotometric readings that the amount of NO released from control macrophages was 20.25 μM which was reduced to 18.25 μM in the cells of adrenalectomised animals. Similarly, the amount of NO released from LPS stimulated cell 19.5 μM was also decreased to 17.25 μM following adrenalectomy ($p \leq 0.01$) (Fig. 3).

Effect of adrenalectomy on the functional integrity of rat peritoneal macrophages – as determined by fragmentation of DNA

The effect of adrenalectomy on the functional integrity of peritoneal Mϕ may be demonstrated particularly from its effect on DNA fragmentation, and also deduce whether adrenal hormone insufficiency may lead to apoptosis. In our study the percentage of DNA fragmentation in control Mϕ was 68.82 ± 4 which was reduced to 56.76 ± 1 following adrenalectomy (Fig. 4). A greater percentage of DNA fragmentation observed in the control cells, probably indicating that endogenous hormone induces apoptosis which was less in adrenalectomised rat due to insufficient endogenous glucocorticoids ($p \leq 0.01$).

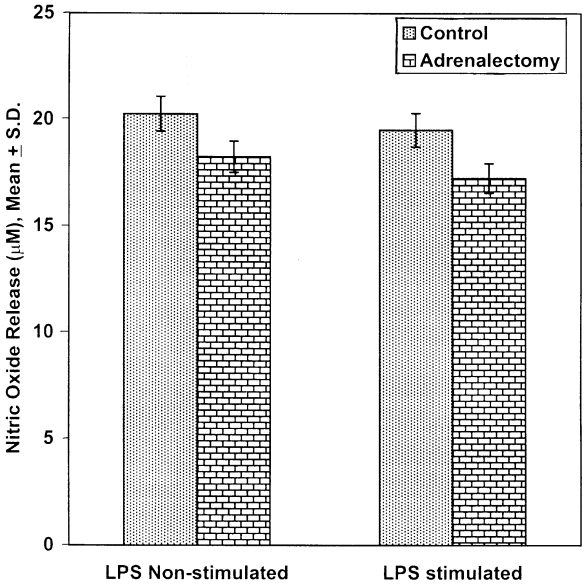

Fig. 3. Effect of adrenalectomy on the nitric oxide release from the rat peritoneal macrophages. The results in this figure represent the nitric oxide released (in μM) and values were expressed as Mean ±S.D. of three independent experiments ($p \leq 0.01$)

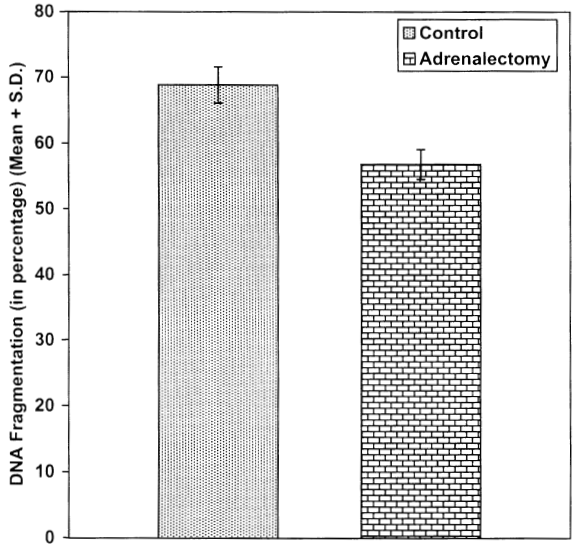

Fig. 4. Effect of adrenalectomy on the functional integrity of rat peritoneal macrophages – as determined by fragmentation of DNA. Results in this figure represent the extent of DNA fragmentation (in percentage) of control and adrenalectomised rat peritoneal macrophages ($p \leq 0.01$)

Effect of adrenalectomy on induction of humoral immune response

Agglutination reaction denotes the interaction of serum antibody with particulate antigen when any bacterial infection occurs in a patient. In adrenalectomised animal high antibody titre (1 : 128) may represent an increased B-cell activity with respect to control group (1 : 32) suggesting that glucocorticoid insufficiency enhances humoral immune response (Table 1).

Table 1
Effect of adrenalectomy on the alteration in antibody titre of serum in rat

	Experimental conditions	Antibody titre of serum
Control	Nonimmunized	No visible agglutination
Control	SRBC immunized	1 : 32
Adrenalectomy	Nonimmunized	No visible agglutination
Adrenalectomy	SRBC immunized	1 : 128

DISCUSSION

Adrenalectomy causes decreased glucocorticoid hormone production as well as release which in turn reduces the normal level of glucocorticoid hormone (GC) in the blood. A significant elevation of phagocytic index in the cells of adrenalectomised animals reflects the increased phagocytic capacity. Normally GC inhibits the growth and activation of Mϕ *(in vivo)* by reducing Granulocyte Macrophages Colony Stimulating Factor (GMCSF) secretion [22] and also block monocyte to macrophage differentiation. In adrenalectomised animal, less secretion of GC may cause proportionate increase in Mϕ function (Fig. 1).

However, partial depletion of endogenous GC by adrenalectomy also reduces ingestion capacity of rat peritoneal Mϕ as it can be seen from the intracellular killing assay. Since cells recovered from adrenalectomised animals are not active enough or somehow are less potent to kill *S. aureus* efficiently, we obtain more viable bacteria in the extracellular supernatant. Whereas control cells are efficiently potent and kill almost all the bacteria intracellularly. As macrophages mature there is progressive rise in lysosomes and in their hydrolytic content. Adrenal hormone insufficiency may also lead to immaturation of macrophages *in vivo,* and it can be assumed that due to adrenalectomy, they have lesser lysosomal content and hence are less capable of digesting bacteria. However, we have not tested this so far. In our study no focus has been made to elucidate the enzyme system interaction for microbial killing after adrenalectomy.

A number of anti-microbial and cytotoxic substances released by activated macrophages are responsible for the intracellular destruction of phagocytosed micro-

organism. When macrophages are activated with bacterial cell wall, lipopolysaccharide, they begin to express high level of nitric oxide synthase which is oxidized L-arginine to yield citrulline and nitric oxide (NO).

In order to further clarify the role of adrenal hormones on immunomodulation we have studied the effect of adrenalectomy on nitric oxide release by the peritoneal Mϕs. Nitric oxide released from peritoneal Mϕs of adrenalectomised rats are less than that of control animal, even when Mϕs are stimulated with LPS. This can be explained by the fact that adrenalectomy not only reduces the secretion of GC from adrenal gland but also results in less secretion of macrophage migration inhibitory factor (MIF) from the cells of the adrenal cortex [27]. MIF is a known costimulator of NO release [17]. Due to adrenalectomy MIF is also depleted which causes reduced NO release even after stimulation with LPS. DNA fragmentation assay shows a lesser degree of fragmentation of peritoneal macrophages in the cells obtained from adrenalectomised rats which indicates apoptotic death of T cells in this group decreases. This study suggests that glucocorticoid (endogenous) has important role in T cell maturation and activation process. In the control group, normal blood GC level helps in negative selection, thus immature T cells are deleted from the circulation. Whereas in adrenalectomised animal immature T cells remain in the circulation due to less glucocorticoid and hence T cell maturation slows down. In such case immature T cells do not undergo apoptotic death as reflected by decreased DNA fragmentation. In order for steady-state levels of the various haematopoietic cells to be maintained, cell division and differentiation are balanced by a process called programmed cell death. Cells undergoing this process often exhibit distinct morphological changes collectively referred to as "apoptosis". This includes pronounced decrease in cell volume, membrane blebbing, condensation of the cytoplasm and chromatin and degradation or fragmentation of DNA. The fragmentation of DNA can, therefore, be considered as one of the indicators of apoptosis. Although programmed cell death is a normal phenomenon to maintain a balance in the number of cells, any modulator which induces DNA fragmentation causes a decrease in the number of viable cells, causing the death of more cells than would be normally required to maintain a balance. This in turn may be harmful to the host.

In agglutination reaction it is observed that adrenalectomy causes increase in antibody raising capacity with respect to control as evident from the high antibody titre in adrenalectomised rat. Since, normally endogenous glucocorticoids are immunosuppressive in nature, when GC levels are depleted by adrenalectomy the immunosuppressive effect of GC in withdrawn. This may lead to increased B cell activity or increase B cell population which in turn enhances the secreted antibody in the serum. So glucocorticoid insufficiency enhances humoral immune response.

Thus on one hand partially glucocorticoid depleted animals show enhanced phagocytic capacity, antibody raising capacity as well as on the other hand it reduces the intracellular killing capacity, NO release, improper cell maturation and heightens the probability of infection. However, the exact mechanism by which adrenalectomy causes the aforesaid immunomodulation is unknown and needs further investigation.

ACKNOWLEDGEMENT

We are thankful to UGC for providing financial assistance to Dr. Biswadev Bishayi, under UGC Minor Research Scheme.

REFERENCES

1. Blalock, J. E. (1994) The syntax of immuno-neuroendocrine communication. *Immunol. Today 15,* 504–511.
2. Casadevell, M., Saperas, E., Panes, J., Salas, A., Anderson, D. C., Malagelada, J. R. (1999) Mechanism underlying the anti-inflammatory action of central corticotropin releasing factor. *Amer. J. Physiol. 276,* G1016–1026.
3. Claman, H. N. (1972) Corticosteroids and lymphoid cells. *N. Engl. J. Med. 287,* 388–397.
4. Cupps, T. R., Fanci, A. S. (1982) Corticosteroid mediated immuno regulation in man. *Immunol. Rev. 65,* 133–155.
5. Czuprynski, C. J., Henson P. M., Campbell, P. A. (1984) Killing of *Listeria monocytogenes* by inflammatory neutrophils and mononuclear phagocytes from immune and non-immune mice. *J. Leukocyte Biol. 35,* 193–208.
6. Da-Silva, J. A., Peers, S. H., Pereretti, M., Willoughby, D. A. (1993) Sex steroids affect glucocorticoid response to chronic inflammation and to interleukin 1. *J. Endocrinol. 136,* 389–397.
7. Dan, G., Lall, S. B. (1998) Neuroendocrine modulation of immune system. *Ind. J. Pharmacol. 30,* 129–140.
8. Gonzalo, J. A., Gonzalez-Garcia, A., Mestinez, C., Kroemer, G. (1993) Glucocorticoid mediated control of the activation and clonal deletion of peritoneal T-cells *in vivo. J. Exp. Med. 177,* 1239–1246.
9. Gruber, J., Sgone, R., Hu, Y. H., Beng, H., Wick, G. (1994) Thymocyte apoptosis by elevated endogenous corticosterone level. *Eur. J. Immunol. 24,* 1115–1121.
10. Jeffries, W. M. (1991) Cortisol and immunity. *Med. Hypothesis 34,* 198–208.
11. Kabat, E. A., Myer, M. M. (1967) Agglutination. In: Kabat, E. A. (ed.) *Experimental Immuno Chemistry,* 2nd ed. 97–132.
12. Leigh, P. C. J., Van Furth, R., Zweet, T. L. (1986) In vitro determination of phagocytosis and intracellular killing by polymorphonuclear neutrophils and mononuclear phagocytes. In: Weir, D. M. (ed.) *Handbook of Experimental Immunology.* 4th ed. Blackwell Scientific Publication, Glasgow, 46.1–46.19.
13. Masferrer, J. L., Reddy, S. T., Zweifel, B. S., Seibert, K., Needleman, P., Gilbert, R. S., Herschman, H. R. (1994) *In vivo* glucocorticoids regulate cyclooxygenase 2 but not cyclooxygenase 1 in peritoneal macrophages. *J. Pharmacol. Exp. Ther. 270,* 1340–1344.
14. Munck, A., Guyre, P. M., Holbrook, N. (1984) Physiological function of glucocorticoid in stress and their relation to pharmacological actions. *Endocrine Rev. 5,* 25–44.
15. Naraba, H., Murakami, M., Matsumoto, H., Shimbara, S., Ueno, A., Kudo, I., Ohishi, S. (1998) Segregated coupling of phospholipase A_2, Cyclooxygenases and terminal prostanoid synthesis in rat peritoneal macrophages. *J. Immunol. 160,* 2974–2982.
16. Nieto, M. A., Gonzalez, F., Ganbon, F., Diaz-Espada, F., Lopez-Rivas, A. (1992) Apoptosis in human monocytes after treatment with glucocorticoids. *Clin. Exp. Immunol. 88,* 341–344.
17. Nishihira, J., Mitchell, R. A., Bucala, R. (2000) Macrophage migration inhibitory factor (MIF): Its essential role in the immune system and cell growth. *J. Interferon. Cytokine. Res. 20,* 751–762.
18. Ochiai, T., Fukushima, K., Ochiai, K. (1997) Butyric acid induced apoptosis of murine thymocytes splenic T-cells. *Infect. Immun. 65,* 35–41.
19. Peers, S. H., Smillie, F., Elderfield, A. J., Flower, R. J. (1993) Glucocorticoid and non-glucocorticoid induction of lipocortins (annexins) 1 and 2 and rat peritoneal leukocytes *in vivo. Br. J. Pharmacol. 108,* 66–72.
20. Plant, M. (1987) Lymphocyte hormone receptors. *Ann. Rev. Immunol. 5,* 621–669.

21. Rook, G., Baker, K., Walker, B., Honour, J. (2000) Local regulation of glucocorticoid activity in sites of inflammation. Insights from the study of tuberculosis. *Amer. N. Y. Acad. Sci. 917,* 913–922.
22. Sakai, M., Biwa, T., Matsumura, T., Takemura, T., Matsuda, H., Anami, Y., Sasahara, T., Kobori, S., Shichiri, M. (1999) Glucocorticoids inhibits oxidized LDL-induced macrophage growth by suppressing the expression of granulocyte macrophage colony stimulating factor. *Arterioscler-Thromb-Vasc. Biol. 19,* 1726–1733.
23. Sasaki, S., Muira, T., Nishikawa, S., Yamada, K., Hirasue, M., Nakane, A. (1998) Protective role of nitric oxide in *S. aureus* infection in mice. *Infect. Immun. 66,* 1017–1022.
24. Sei, T. (1992) Hormonal regulation of the immune response in stress, immunity and aging. In: E. L. Cooper (ed.) Stress, immunity and aging. Mercell Dekker Inc. New York, pp. 141–156.
25. Shimoji, Y., Yokomizo, Y., Sekiazi, T., Mori, Y., Kubo, M. (1994) Presence of a capsule in *Erysiophelothrix rhusiopathiae* and its relationship to virulence for mice. *Infect. Immun. 62,* 2806–2810.
26. Silverstein, R. (1991) Hydrazin sulfate protects D-galactosamine sensitized mice against endotoxin and tumor necrosis factor/cachectin lethality. Evidence of a role for the pituitary. *J. Exp. Med. 173,* 357–365.
27. Thierry, C., Jurgen, B., Christine, N. M. (1995) MIF as a glucocorticoid induced modulator of cytokine production. *Nature 377,* 68–71.
28. Vacchio, M. S., Lee, J. Y., Ashwell, J. D. (1999) Thymus derived glucocorticoids set the thresholds for thymocyte selection by inhibiting TCR mediated thymocyte activation. *J. Immunol. 163,* 1327–1333.
29. Vacchio, M. S., Papadopoulos, V., Ashwell, J. D. (1994) Steroid production in the thymus: implication for thymocyte selection. *J. Exp. Med. 179,* 1835–1846.
30. Wick, G., Hu, Y., Schwarz, S. (1993) Immunoendocrine communication via the hypothalamo-pituitary adrenal axis in autoimmune diseases. *Endocrine Rev. 14,* 539–563.
31. Wilder, R. L. (1995) Neuroendocrine-immune system interaction and autoimmunity. *Ann. Rev. Immunol. 13,* 307–338.