Suppressive effect of 1,25(OH)2D3, and glucocorticoids on production of tumor necrosis factor-alpha by human peripheral blood adherent cells.

Katakami, N

Nakao, Y

Fujita, T

(Citation)
The Kobe journal of the medical sciences,37(3):179-188

(Issue Date)
1991

(Resource Type)
departmental bulletin paper

(Version)
Version of Record

(URL)
https://hdl.handle.net/20.500.14094/E0034102

Kobe J. Med. Sci. 37, 179/188, June 1991

SUPPRESSIVE EFFECT OF $1,25(OH)_2D_3$, AND GLUCOCORTICOIDS ON PRODUCTION OF TUMOR NECROSIS FACTOR-α BY HUMAN PERIPHERAL BLOOD ADHERENT CELLS

Nobuyuki KATAKAMI, Yosinobu NAKAO, AND Takuo FUJITA

Third Division, Department of Medicine, Kobe University School of Medicine, Kobe, 650, Japan

INDEXING WORDS

tumor necrosis factor- α; $1,25(OH)_2D_3$; glucocorticoid; human adherent cell; ELISA

SYNOPSIS

Effect of $1,25(OH)_2D_3$ and glucocorticoids on production of TNF-α by human peripheral blood adherent cells (HPBAC) was investigated. TNF-α was measured by ELISA method using monoclonal antibody against human recombinant THF-α. Maximal TNF- α production by HPBAC was observed in 4 to 8 hr after incubation when these cells were cultured for 24 hr with 10 μg/ml lipopolysaccharide (LPS), 10ng/ml 12-o-tetradecanoyl-phorbol-13-acetate (TPA) and 10^{-5}M indomethacin (INDM). When graded concentration of $1,25(OH)_2D_3$ was added to HPBAC in the presence of above stated dose of LPS, TPA and INDM, a significant suppression of TNF-α production was first observed at the concentration of 10^{-8}M ($P<0.05$). Similar significant suppressive effect was also observed by adding hydrocortisone and dexamethasone at the concentration of 10^{-8}M ($P<0.05$) and 10^{-7}M ($P<0.05$), respectively. These results may suggest that $1,25(OH)_2D_3$ has the same inhibitory effect on monocyte/macrophage function as glucocorticids.

Received for publication: June 27, 1991
Author's name in Japanese: 片上信之, 中尾實信, 藤田拓男

INTRODUCTION

Tumor necrosis factor (TNF), which was found originally in mouse serum after intravenous injection of bacterial endotoxin into mice primed with viable *Mycobacterium bovis*, is one of the monokines produced by macrophages and monocytes.[7,18] TNF has a characteristic property that induces hemorrhagic necrosis in certain animal tumors and in some heterotransplanted human tumors, but it seems to be innocuous to normal cells *in vitro*. Recently, human TNF has been purified, sequenced and cloned by recombinant DNA methods.[12,23] Accordingly, a number of studies have been undertaken to investigate the mechanism of action, other roles besides causing hemorrhagic necrosis in tumors and interactions with important substances such as interleukin 2 and interferons.[4,20,24,26]

In addition to acting as a hormone in calcium and bone homeostasis, $1,25(OH)_2D_3$ is well known to have immunoregulatory functions and plays an important role in cell replication and differentiation.[1,15,17,21] At present relatively little is known about interactions between TNF and this active vitamin.[22] In the present study, effect of $1,25(OH)_2D_3$ on the production of TNF by human peripheral blood adherent cells was evaluated and compared with that of glucocorticoids.

MATERIALS AND METHODS

Culture medium and reagents.

RPMI 1640 medium (M.A. Bioproducts) was supplemented with 5% heat inactivated fetal calf serum, L-glutamine (2 mM), Hepes (0.025 M), and kanamycin (0.12 mg/ml). Lipopolysaccharide (LPS) was purchased from Difco. 12-o-tetradecanoyl-phorbol-13-acetate (TPA) was obtained from Sigma Chemical Co. (St. Louis, MO). $1,25(OH)_2D_3$ was a gift of Teijin Institute for Biomedical Research (Tokyo, Japan).

Peripheral blood adherent cells and culture.

Peripheral venous blood from healthy adult volunteers was sedimented on Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ) gradients. Mononuclear cell layer was removed and washed three times with RPMI 1640 medium containing 5% heat inactivated FCS. Mononuclear cells were resuspended in RPMI 1640 medium, 5% FCS at a final concentration of 1×10^7 cells/ml, then redistributed at 1×10^7 cells/well in polystyrene 24-well culture plates (Corning, New York) and incubated for 1 hr at 37℃ in 5% CO_2-95% air. Nonadherent cells were removed by washing vigorously 3 times with cooled (4 ℃) RPMI 1640 medium. The cells were incubated for various hours for the first 24 hr at 37℃ in 5% CO_2 -95% air with 1 ml of RPMI 1640 medium containing 5% heat inactivated FCS in the presence of LPS (10μg/ml), TPA (10ng/ml) and INDM (10^{-5}M). This experiment was done to deterimne optimal incubation time for TNF-α production by adherent cells. The maximal

TNF production was measured between 4 to 8 hr after the initial exposure to LPS, TPA and INDM (data not shown). After this preliminary experiment the cells were incubated for 6 hr with various concentration of $1,25(OH)_2D_3$, dexamethasone or hydrocortisone in the presence of LPS (10μg/ml), TPA (10ng/ml) and INDM (10^{-5}M). After incubation, the cell suspensions were centrifuged and cell free supernatants were harvested and stored at -20 ℃ until assayed.

TNF assay.

Human adherent cell supernatants were assayed for their TNF-α content by using enzyme-linked immunosorbent assay (ELISA). Briefly, one monoclonal antibody (the first antibody) was immobilized in the wells of 96-well microplates. After washing to remove excess antibody, the standard and test samples were reacted with the immobilized first antibody. After washing to remove excess test sample, the second monoclonal antibody conjugated with peroxidase (the second antibody) was reacted. After washing to remove excess second antibody, substrate (O-phenylenediamine) was added and the absorbance of the product was measured to determine the concentration of TNF-α in the test samples. Standard human recombinant TNF-α and anti-TNF-α monoclonal antibody were kindly supplied by Asahi Chemical Industry (Tokyo, Japan).

Statistical analysis.

Statistical significance of differences between groups of experiments was assessed by Student's t test. Confidence level was at $P<0.05$.

RESULT

Effect of $1,25(OH)_2D_3$ on TNF production. Fig. 1 represents the results of an experiment examining the effect of $1,25(OH)_2D_3$ on TNF production when graded concentration of $1,25(OH)_2D_3$ was added to adherent cells in the presence of LPS, TPA and INDM. A significant suppression of TNF production was first observed at the concentration of 10^{-8}M ($P<0.05$). This suppressive effect seems to be dose-dependent, whereas physiological concentrations of $1,25(OH)_2D_3$ (10^{-10}-10^{-11}M) did not suppress TNF production.

Effects of Hydrocortisone and Dexamethasone on TNF production. A meaningful suppression of TNF production by hydrocortisone was first observed at 10^{-8}M ($P<0.05$). This effect also followed in a dose-dependent fashion. More importantly, TNF production was suppressed at a physiological concentration of hydrocortisone (10^{-7}M) (Fig. 2). Similar inhibitory effect was also noted with dexamethasone, although it occurred at a higher concentration (10^{-7}M) and was reproducible (Fig. 3).

DISCUSSION

It is currently noted that there are two kinds of TNFs, TNF-α and TNF-β, which display cytolytic and/or cytotoxic activities against a wide range of tumor cell.[2,19] In our study, TNF production by adherent cells is assayed by ELISA method using specific monoclonal antibody against human recombinant TNF-α. Consequently supernatent TNF detected in our assay system represents TNF-α.

It is clear that TNF production by adherent cell is augmented by various stimulants

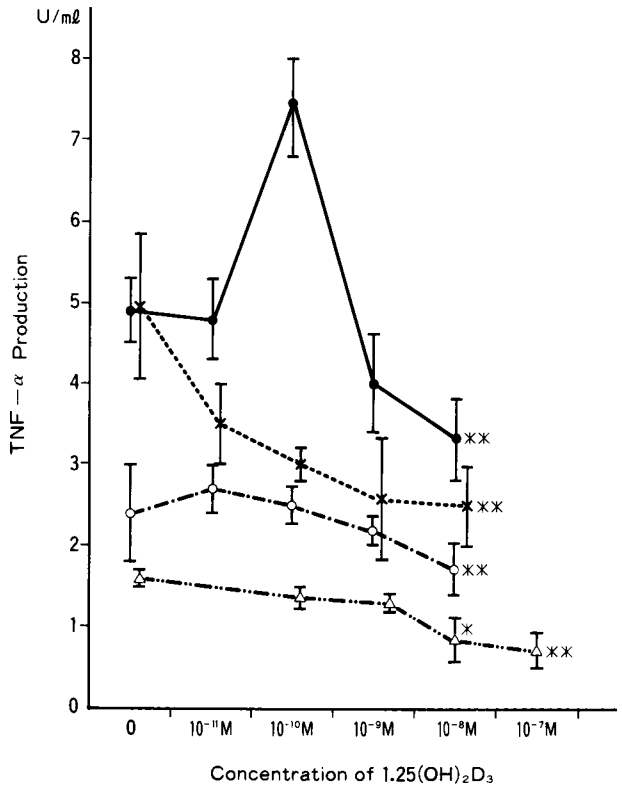

Fig.1. Effect of $1,25(OH)_2D_3$ on TNF-α production by human peripheral blood adherent cells. When graded concentration of $1,25(OH)_2D_3$ was added to adherent cells from four normal human volunteers in the presence of LPS (10μg/ml), TPA (10ng/ml) and INDM (10^{-5}M), a significant suppression of TNF-α production was first observed at the concentration of 10^{-9}M. Each value represents the mean $\pm$ SD of triplicate cultures. Statistical sifnificance of a difference from $1,25(OH)_2D_3$ absent medium is indicated as (*) $P<0.05$ and (**) $P<0.01$. All standard deviations were less than 5% of the mean values.

such as LPS and TPA.[3,20,29] On the other hand, prostaglandin E_2 is reported to inhibit TNF production by macrophages,[16] and is synthesized by monocyte/macrophages when the cells are cultured with various stimulants.[9] Therefore we added indomethacin to each culture medium to counteract the effect of prostaglandin E_2 on adherent cells.

TNF activity appeared in adherent cell supernatants after 2 hr and peaked after 4 to 8 hr and longer incubation led to a loss of activity. This result accords with the experiment carried out by Chen *et al*[5] demonstrating that LPS stimulated cytolytic monokine production by human peripheral monocytes peaked after 4 to 7 hr incubation and then declined despite the continued presence of LPS.

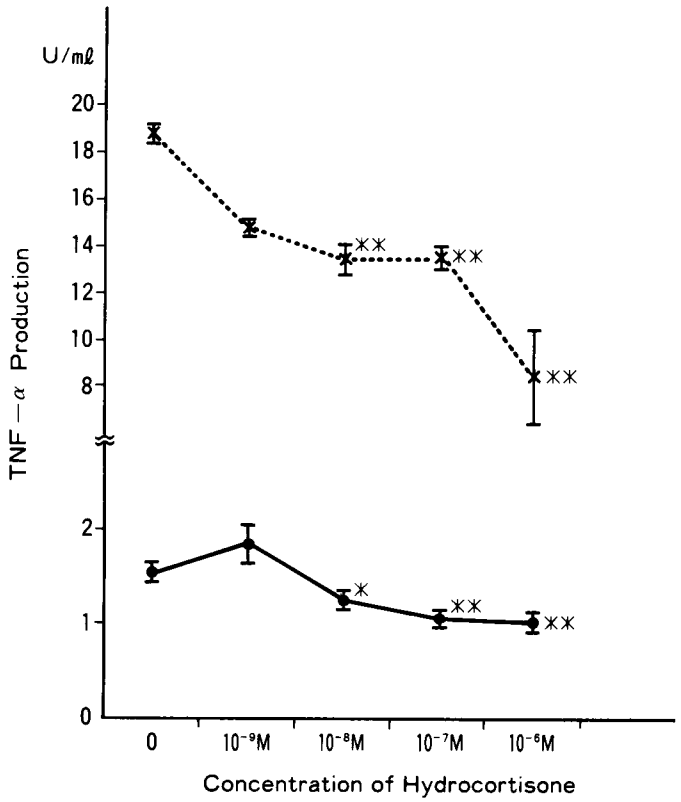

Fig.2. Effect of hydrocortisone on TNF-α production by human peripheral blood adherent cells. When graded concentration of hydrocortisone was added to adherent cells from two normal human volunteers in the presence of LPS (10μg/ml), TPA (10ng/ml) and INDM (10^{-4}M), a significant suppression of TNF-α production was first observed at the concentration of 10^{-7}M. Each value represents the mean $\pm$ SD of triplicate cultures. Statistical significance of a difference from hydrocortisone absent medium is indicated as (*) $P<0.05$ and (**) $P<0.01$.

There was considerable variation in baseline TNF production by adherent cells among donors. Invariable, however, addition of $1,25(OH)_2D_3$, dexamethasone or hydrocortisone to adherent cell culture resulted in a significant suppression of TNF synthesis. Recent evidence demonstrated that glucocorticoids inhibit interleukin 1 and interleukin 2 production which is a function of macrophages and T lymphocytes, respectively.[11,13,25] Furthermore, glucocorticoids has been reported to inhibit the LPS-induced production of TNF not only by murine macrophages[5] but also by human peripheral monocytes.[10,28] Our data also confirmed this suppressive effect of glucocorticoids. While the immunoregulatory role of glucocorticoids has been recognized since their discovery in 1948, but their mechanism of action is still not clear

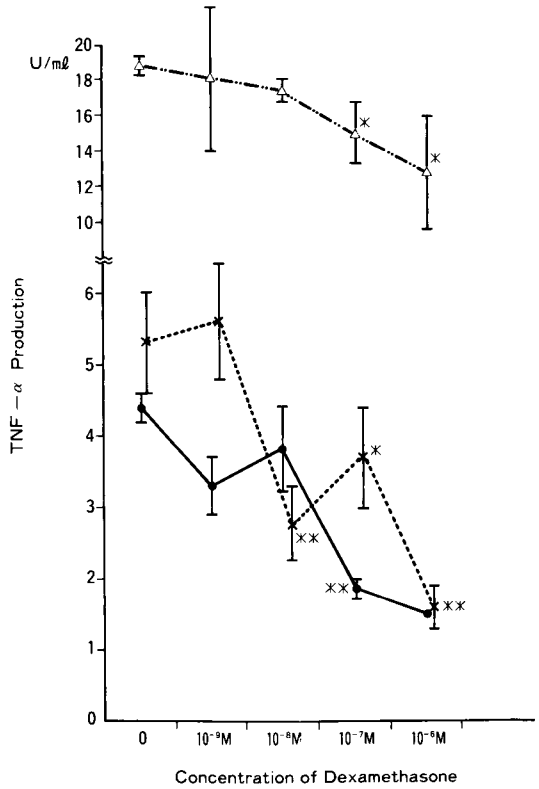

Fig.3. Effect of dexamethasone on TNF-α production by human peripheral blood adherent cells. When graded concentrations of dexamethasone were added to adherent cells from three normal human volunteers in the presence of LPS (10μg/ml), TPA (10ng/ml) and INDM (10^{-5}M), a significant suppression of TNF-α production was first observed at the concentration of 10^{-8}M. Each value represents the mean ± SD of triplicate cultures. Statistical significance of a difference from dexamethasone absent medium is indicated as (*) $P<0.05$ and (**) $P<0.01$.

and is now actively under investigation.

Recent studies have demonstrated that the hormonal form of vitamin D_3, $1,25(OH)_2D_3$, has immunoregulatory properties besides its classical function as a regulator of calcium metabolism. $1,25(OH)_2D_3$ inhibits interleukin 2 production by human peripheral T lymphocytes activated by the phytohem-agglutinin (PHA) and is a potent inhibitor of PHA-induced lymphocyte blast transformation.[27] On the other hand, there are conflicting experimental results as to the effect of $1,25(OH)_2D_3$ on interleukin 1 (IL-1) production by peripheral human monocyte/macrophages: inhibition of IL-1 production[14] and augumentation of IL-1 production.[6] As far as TNF production by human peripheral adherent cells is concerned, its enhanced release has been described following 3-5 days incubation of monocytes with 10^{-7}M $1,25(OH)_2D_3$ in the presence of LPS for the last 16 hr in the fresh medium by Rook *et al.*[21] Their data seems to be against our result. However, their experimental condition is quite different from ours. Firstly, they incubated adherent cells overnight before adding $1,25(OH)_2D_3$ in the medium. Secondary, and more importantly, they preincubated adherent cells with $1,25(OH)_2D_3$ more than 2 days before exposure to LPS for 16 hr in fresh medium. Therefore adherent cells might get more matured and differentiated. In contrast to this, we did not incubate adherent cells after removing non-adherent cells and incubated adherent cells thgether with LPS, TPA, INDM and $1,25(OH)_2D_3$ for much shorter period (5.5hr).

Physiological concentration of $1,25(OH)_2D_3$ (10^{-10}-10^{-11}M) does not suppress TNF production. Actually, TNF production seems to be enhanced at the concentration of 10^{-10}M in one out of 4 experiments. Although the exact mechanism of this phenomenon is not clear, $1,25(OH)_2D_3$ might maturate and differentiate adherent cells and more mature cells could produce significant amount of TNF. Anyway in our assay system we have clearly demonstrated that TNF-α is suppressed by $1,25(OH)_2D_3$ at the con- centration of 10^{-8}M and higher. Several possibilities can account for the inhibition of TNF production: 1) $1,25(OH)_2D_3$ may exert its effect directly on TNF by inhibiting its production or secretion, or both; 2) $1,25(OH)_2D_3$ may suppress monocyte proliferation or differentiation through its receptors on these cell, which would result in turn in decreased production of TNF; 3) $1,25(OH)_2D_3$ may induce the monocyte to release a factor with direct inhibitory effects on TNF activity. We have presented evidence that TNF production by adherent cells is suppressed not only by glucocorticoids but also by $1,25(OH)_2D_3$ and this result is appreciable to understand immunopharmacological basis for clinical use of the hormones. Precise mechanisms of actions of these hormones against immunocompetent cells are under investigation in our laboratory.

ACKNOWLEDGMENTS

We thank Miss Kyoko Tanaka for her excellent technical assistance in preparing

peripheral blood adherent cells. This investigation is supported in part by a Grant-in-Aid from Kobe City General Hospital, Kobe, Japan.

REFERENCES

1. Abe, E., Miyaura, C., Sakagami, H., Takeda, M., Konno, K., Yamazaki, T., Yoshiki, S. and Suda, T.: Proc. Natl. Acad. Sci. U.S.A. 1981. 78. 4990/4994. Differentiatio of mouse myeloid leukemia cells induced by 1,25-dihydroxyvitamin D_3.
2. Aggarwal, B.B., Eessalu, T.E. and Hass, P.E.: Nature. 1985. 318. 665/667. Characterization of receptors for human tumour necrosis factor and their regulation by γ -interferon.
3. Aggarwal, B.B., Kohr, W.J., Hass, P.E., Moffat, B., Spencer, S.A., Henzel, W.J., Bringman, T.S., Nedwin, G.E., Goeddel, D.V. and Harkins, R.N.: J. Biol. Chem. 1985. 260. 2345/2354. Human tumor Necrosis factor: Production, purification and characterization.
4. Bertolini, D.R., Nedwin, G.E., Bringman, T.S., Smith, D.D. and Mundy, G.R.: Nature. 1986. 319. 516/518. Stimulation of bone formation *in vitro* by human tumour necrosis factors.
5. Beutler, B., Krochin, N., Milsark, I.W., Luedke, C. and Cerami, A.: Science. 1986. 232. 977/980. Control of cachectin (tumor necrosis factor) synthesis: Mechanisms of endotoxin resistance.
6. Bhalla, A.K., Amento, E.P. and Krane, S.M.: Cell. Immunol. 1986. 98. 311/322. Differential effects of 1,25-dihydroxy-vitamin D_3 on human lymphocytes and monocyte/macrophages: Inhibition of interleukin-2 and augmentation of interleukin-1 production.
7. Carswell, E.A., Old, L.J., Kassel, R.L., Green, S., Fiore, N., and Williamson, B.: Proc. Natl. Acad. Sci. USA. 1975. 72. 3666/3670. An endotoxin-induced serum factor that causes necrosis of tumors.
8. Chen, A.R., Mckinnon, M.P. and Koren, H.S.: J. Immunol. 1985. 135. 3978/3987. Lipopolysaccharide (LPS) stimulates fresh human monocytes to lyse actinomycin D-treated WEHI-164 target cells via increased secretion of a monokine similar to tumor necrosis factor.
9. Davies, P., Bailey, P.J. and Goldenberg, M.M.: Ann. Rev. Immunol. 1984. 2. 335/357. The role of arachidonic acid oxygenation products in pain and inflammation.
10. Debets, J.M.H., Ruers, T.J.M., Van Der linden, M.P.M.H., Vander Linden, C.J. and Buurman, W.A.: Clin. Exp. Immunol. 1989. 78. 224/229. Inhibitory effect of corticosteroids on the secretion of tumour necrosis factor (TNF) by monocytes is dependent on the stimulus inducing TNF synthesis.

11. Goodwin, J.S., Atluru, D., Sierakowski, S. and Lianos, E.A.: J. Clin. Invest. 1986. 77. 1244/1250. Mechanism of action of glucocorticosteroids: Inhibition of T cell proliferation and interleukin 2 production by hydrocortisone is reversed by leukotriene B_4.
12. Gray, P.W., Aggarwal, B.B., Benton, C.V., Bringman, T.S., Henzel, W.J., Jarrett, J.A., Leung, D.W., Moffat, B., Svedersky, P.N.L.P., Palladino, M.A. and Nedwin, G.E.: Nature. 1985. 312. 721/724. Cloning and expression of cDNA for human lymphotoxin, a lymphokine with tumour necrosis activity.
13. Grujicic, S.S. and Simic, M.M.: Cell. Immunol. 1982. 69. 235/247. Modulation of interleukin 1 production by activated macrophages: In vitro action of hydrocortisone, colchicine and cytochalasin B.
14. Iho, S., Kura, F., Sugiyama, H., Takahashi, T. and Hoshino, T: Immunol. Lett. 1985. 11. 331/336. The role of monocytes in the suppression of PHA-inducedproliferation and IL 2 production of human mononuclear cells by 1,25 dihydroxyvitamin D_3.
15. Koizumi, T., Nakao, Y., Matsui, T., Nakagawa, T., Matsuda. S., Komoriya, K.,Kanai, Y. and Fujita, T.: Int. Archs. Allergy. Appl. Immun. 1985. 77. 396/404. Effects of corticosteroid and 1,24R-dihydroxy-vitamin D_3 administration on lymphoproliferation and autoimmune disease in MRL/MP-1pr/1pr mice.
16. Kunkel, S.L., Wiggins, R.C., Chensue, S.W. and Larrick, J.: Biochem. Biophys. Res. Comm. 1986. 137. 404/410. Regulation of macrophage tumor necrosis factor production by prostaglandin E_2.
17. Matsui, T., Nakao, Y., Koizumi, T., Nakagawa, T. and Fujita, T.: Life. Sciences. 1985. 37. 95/101. 1,25-dihydroxyvitamin D_3 regulates proliferation of activated T-lymphocyte subsets.
18. Matthews, N. and Watkins, J.F.: Br. J. Cancer. 1978. 38. 302/309. Tumor-necrosis factor from the rabbit. I. Mode of action, specificity and physicochemical properties.
19. Nedwin, G.E., Svedersky, L.P., Bringman, T.S., Palladino, M.A.J. and Goeddel, D.V.: J. Immunol. 1985. 135. 2492/2497. Effect of interleukin 2, interferon- γ and mitogens on the production of tumor necrosis factors α and β.
20. Old, L.J.: Science. 1985. 230. 943/945. Tumor necrosis factor (TNF).
21. Rigby, W.F.C., Noelle, R.J., Krause, K. and Fanger, M.W.: J. Immunol. 1985. 135. 2279/2286. The effects of 1,25-dihydroxy-vitamin D_3 on human T lymphocyte activation and proliferation: A cell cycle analysis.
22. Rook, G.A.W., Taverne, J., Leveton, C. and Steele, J.: Immunology. 1987. 62. 229/234. The role of gammainterferon, vitamin D_3 metabolites and tumour necrosis factor in the pathogenesis of tuberculosis.
23. Shirai, T., Yamaguchi, H., Ito, H., Todd, C.W. and Wallace, R.B.: Nature. 1985. 313. 803/806. Cloning and expression in *Escherichia coli* of the gene for human tumour necrosis factor.

24. Slungaard, A., Vercellotti, G.M., Walker, G., Nelson, R.D. and Jacob, H.S.: J. Exp. Med. 1990. 171. 2025/2041. Tumor necrosis factor α/cachectin stimulates eosinophil oxidant production and toxicity towards human endothelium.
25. Snyder, D.S. and Unanue, E.R.: J. Immunol. 1982. 129. 1803/1805. Corticosteroids inhibit murine macrophage Ia expression and interleukin 1 production.
26. Sugarman, B.J., Aggarwal, B.B., Hass, P.E., Figari, I.S., Palladino, M.A.J. and Shepard, H.M.: Science. 1985. Recombinant human tumor necrosis factor-α: Effects on proliferation of normal and transformed cells *in vitro*.
27. Tsoukas, C.D., Provvedine, D.M. and Manolagas, S.C.: Science 1984. 224. 1438/1440. 1,25-dihydroxyvitamin D3: A novel immunoregulatory hormone.
28. Waage, A. and Bakke, O: Immunology. 1988. 63. 299/302. Glucocorticoids suppress the production of tumour necrosis factor by lipopolysaccharide-stimulated human monocytes.
29. Williamson, B.D., Carswell, E.A., Rubin, B.Y., Prendergast, J.S. and Old, L.J.:Proc. Natl. Acad. Sci. U.S.A. 1983. 80. 5397/5401. Human tumor necrosis factor produced by human B-cell lines: Synergistic cytotoxic interaction with human interferon.