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A KINETIC STUDY ON PRODUCTION OF CYTOKINES BY PERIPHERAL BLOOD CELLS

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ABSTRACT

Cytokines are large group of low molecular weight that are polypeptides or glycopeptides in nature and are essential mediators of inflammation and immune reactions. The estimation of cytokine production after cell stimulation is used for understanding the immune status of an individual and thereby in diagnosis and prognosis of diseases. The kinetic study, on several cytokine at one platform, for production of cytokines is scanty. Hence, we have attempted a kinetic study (in terms of dose of stimulants for pro and anti inflammatory cytokines and their time of production) of various cytokines by whole blood cell culture. After stimulating blood cells with phytohaemagglutinin (PHA-L) or lippolysaccharide (LPS) as stimulant, various cytokines {IL-1b, IL-12p70, IL-2, IL-4, IL-10, and tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ)} were estimated in cell culture supernatants by flow cytometry. We have observed that for production of IL-1b, IL-12p70, IL-2, IL-4, IL-6, IL-10, TNF-α and IFN-γ optimal concentration of PHA were 8µg/ml, 16µg/ml, 16µg/ml, 16µg/ml, 8µg/ml, 16µg/ml, 16µg/ml and 8µg/ml respectively. On the other hand, the optimal time for production of these cytokines were found to be 120 hours, 48 hours, 24 hours, 144 hours, 120 hours, 48 hours, 24 hours and 96 hours respectively. Regarding LPS as stimulant, the optimal concentration of LPS for production of IL-1b, IL-12p70, IL-2, IL-4, IL-6, IL-10, TNF-α and IFN-γ were 8µg/ml, 8µg/ml, 8µg/ml, 8µg/ml, 16µg/ml, 4µg/ml, 4µg/ml and 4µg/ml respectively; whereas the optimal timings for production were 96 hours, 96 hrs, 48 hours, 48 hours, 96 hours, 48 hours, 24 hours and 120 hours respectively. These findings would be of relevance in understanding the status of cytokine production in health and diseases.

KEYWORDS

phytohaemagglutinin, lippolysaccharide, flow cytometry

INTRODUCTION

Cytokines are soluble glycoproteineous nonimmunoglobulin biomolecules secreted by living cells of the host, which act nonenzymatically in subnanomolar concentration through specific receptors expressed on target cells.[1,2,3] They constitute the fourth major class of soluble intracellular signaling molecules (inter cellular messenger for cell to cell communication) along with endocrine hormones, growth factors and neurotransmitters.[4, 5]

Cytokines are produced by a large variety of cells. Non lymphoid cells include macrophages, dendrite cells, fibroblasts, keratinocytes, endothelial cells and variety of transformed cell lines. Among lymphoid cells, TH cells and their TH1 and TH2 subpopulations and Tc cells are the producers of cytokines. Overall, TH cells including TH1 & TH2 subsets and macrophages are the principal cytokine producing cells. Cytokine network determines the development and differentiation of TH1 and TH2 subsets of TH cells.[8,11]

In-vivo cells are exposed to a mixture of cytokines having pleiotropic, synergistic redundancy or antagonistic effects which may cause varied types of results.[10] Besides this, cytokines also induce synthesis of other cytokines, which in turn may have inhibitory/ stimulatory effect activity on earlier cytokines.[12] Moreover, some cytokine may be produced by different types of cells and may have different target cells which under the influence of same cytokine express biological activity differently.

The cytokines create cascade like chain activity and complexity in functional network of cytokines. They form central coordinating network of soluble effecter molecules which play a pivotal role in each phase of homoeostasis of the body.[16,17,18]

The estimation of cytokine production after cell stimulation is used for understanding the immune status of an individual and thereby in diagnosis and prognosis of diseases.[15,19] The kinetic study, on several cytokine at one platform, for production of cytokines is scanty.[13,14] Hence, we have attempted a kinetic study (in terms of dose of stimulants for pro and anti inflammatory cytokines and their time of production) of various cytokines by whole blood cell culture

MATERIAL AND METHODS

(i) ACQUISITION OF REAGENTS:

(a)Phytohaemagglutinin (PHA-L) and Lippopolysaccharide (LPS) - Sigma
(b) RPMI 1640 tissue culture media -Sigma
(c) Protease inhibitor cocktail –Sigma
(d) Heparin – Sigma
(e) Antibiotic antimycotic solution - Sigma
(f) Cytometric Bead Array (CBA) kits – BD Biosciences
(g) Cytometric Bead Array (CBA) flex kit – BD Biosciences

(ii) PRODUCTION OF NATIVE CYTOKINE BY WHOLE BLOOD CULTURE:

After obtaining written consent, the blood (5ml) from each healthy individuals was drawn by antecubital venipuncture and collected into heparin containing tubes. All the subjects included in the study were screened for HIV

infection to rule out HIV positive infection. The blood samples were stimulated by various concentrations (0µg/ml, 1µg/ml, 2µg/ml, 4µg/ml, 8µg/ml, 16µg/ml) of phytohaemagglutinin (PHA-L) or lippopolysaccharide (LPS) to know optimum concentration of stimulant. The cell culture supernatants were harvested after 48 hrs and stored after adding protease inhibitor cocktail at -20^{0}C. The blood samples were stimulated by 5 µg/ml PHA or LPS and harvested for the cell culture supernatants after various incubation time period (0 hrs, 24 hrs, 48 hrs, 72 hrs, 96 hrs, 120 hrs, 144 hrs,) to know optimum incubation period. The harvested cell culture supernatants were stored after adding protease inhibitor cocktail at -20^{0}C.

(iii) ESTIMATION OF CYTOKINES:

The estimation of cytokines was done by Cytometric Bead Array (CBA) kit and CBA flex kit using flow cytometer 'FACSAria' (BD Biosciences) according to the instructions

RESULT

A. IL-1b:

IL-1b has shown the dose dependency as the concentration of LPS or PHA increased. The concentration of IL-1b increased up to 8µg/ml concentration of PHA/ LPS and then decline. The concentration (8µg/ml) PHA/ LPS was found to be optimum for maximum production in 48 hrs for IL-1b 1763.96 pg/ml /141.51 pg/ml respectively but differ in magnitude.

When stimulated with LPS (5µg/ml), IL-1b elevated to the level of concentration of 1786 pg/ml within 24 hrs and reached to peak 2385.47 pg/ml in 96 hrs while with PHA (5µg/ml), it elevated to concentration of 180.06 pg/ml and to peak level 238.09 pg/ml in 120 hrs.

B. IL-12p70 :

IL-12-p70 reached peak 4.03 pg/ml and 2.7 pg/ml with LPS (8µg/ml) and PHA (16µg/ml) respectively. The optimum time for maximum production was 96 hrs (6.26 pg/ml) and 48 hrs (3.32 pg/ml) for PHA/LPS respectively.

C. IL-2:

When stimulated with PHA, the produced concentration of IL-2 showed the dose dependency and reached the peak 7518.97 pg/ml at 16 µg/ml concentration of stimulant. On the other hand with LPS, IL-2 peaked at 32.23 pg/ml at 8µg/ml concentration of stimulant. When stimulated with PHA, the concentration of IL-2 reached peak of 450.03 within 24 hrs and sharply declined. With LPS, The peak concentration of IL-2 was 29.13 pg/ml in 48 Hrs and then decrease.

D. IL-4:

IL-4 showed the dose dependency for both PHA/LPS. PHA (16µg/ml) and LPS (8µg/ml) were optimum production 673.63 pg/ml, 600.3 pg/ml of IL-4 respectively. The concentration of IL-4 reached 523.9 pg/ml within 24 hrs and peaked at 574.27 pg/ml at 144 hrs of stimulation with PHA. With LPS, the concentration level of IL-4 was 472.83 pg/ml within 24 hrs and reached peak 547.73 pg/ml in 48 hrs and remained on average 486.19 pg/ml up to 144 hrs of incubation.

E. IL-6:

The concentration of IL-6 produced showed the dose dependency as the concentration of LPS increased. While the concentration of 8µg/ml of PHA was found to optimum for IL-6 production. The optimum time for IL-6 production were 96 hrs (234991.8 pg/ml) and 120 hrs (184482.8 pg/ml) for LPS and PHA respectively.

F. IL-10:

In the case PHA, the concentration of IL-10 produced showed the dose dependency of stimulant. The optimum concentration of PHA and LPS were found to be 4 µg/ml and 16

µg/ml respectively. The 48 hrs of incubation was found to be optimum for both LPS and PHA.

G. TNF-a:

The optimum dose of PHA and LPS were found to be 16µg/ml and 4µg/ml respectively.
The optimum time for TNF-a production was found to be 24 hrs but differ in magnitude 6371.8 pg/ml with LPS and 1423.47 pg/ml with PHA.

H. IFN-Y

The optimum dose of PHA and LPS were found to be 8µg/ml and 4µg/ml respectively. The optimum time for IFN-y production was found to be 96 hrs with PHA and 120 hrs with LPS.

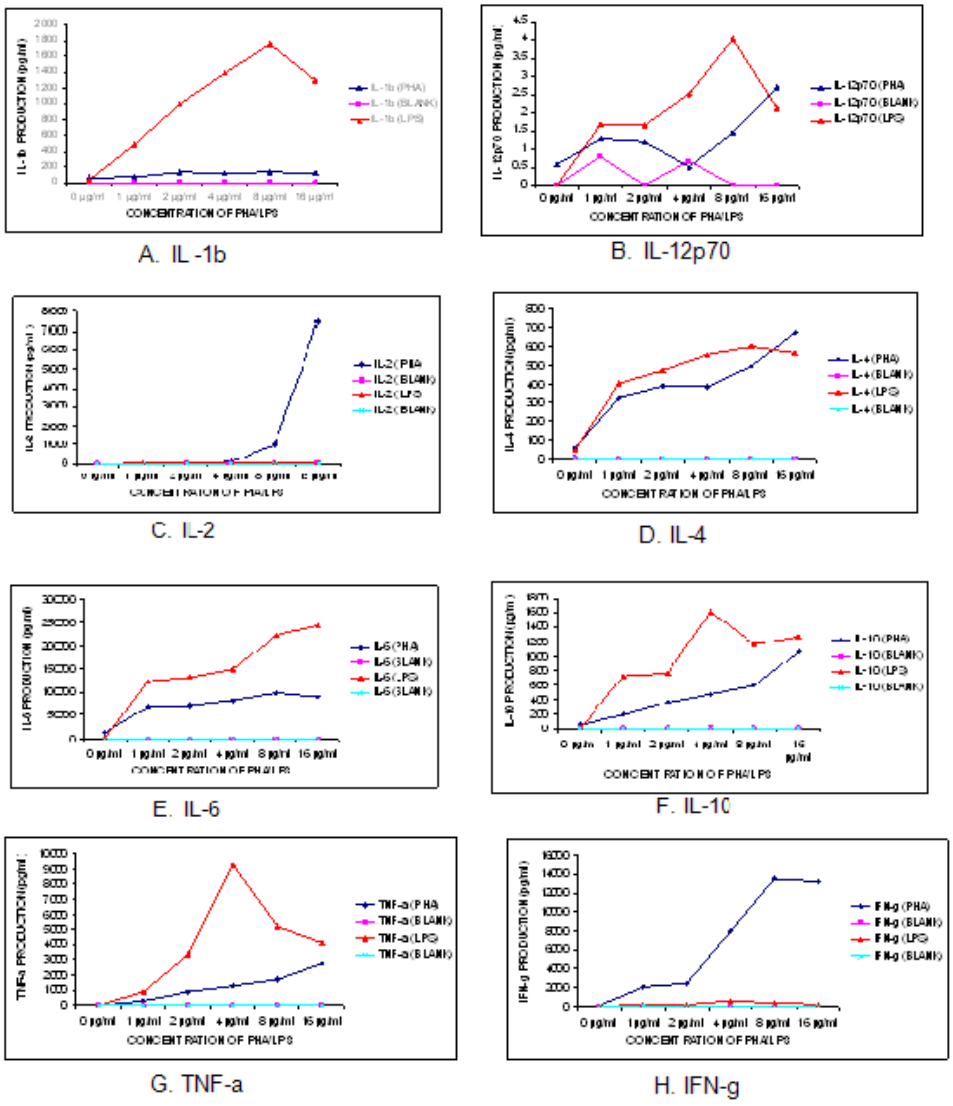

FIGURE 1
CYTOKINE PRODUCTION EMPLOYING VARIOUS CONCENTRATIONS OF PHYTOHAEMAGGLUTININ (PHA) AND LIPPOPOLYSACCHARIDE (LPS) AS A STIMULANT

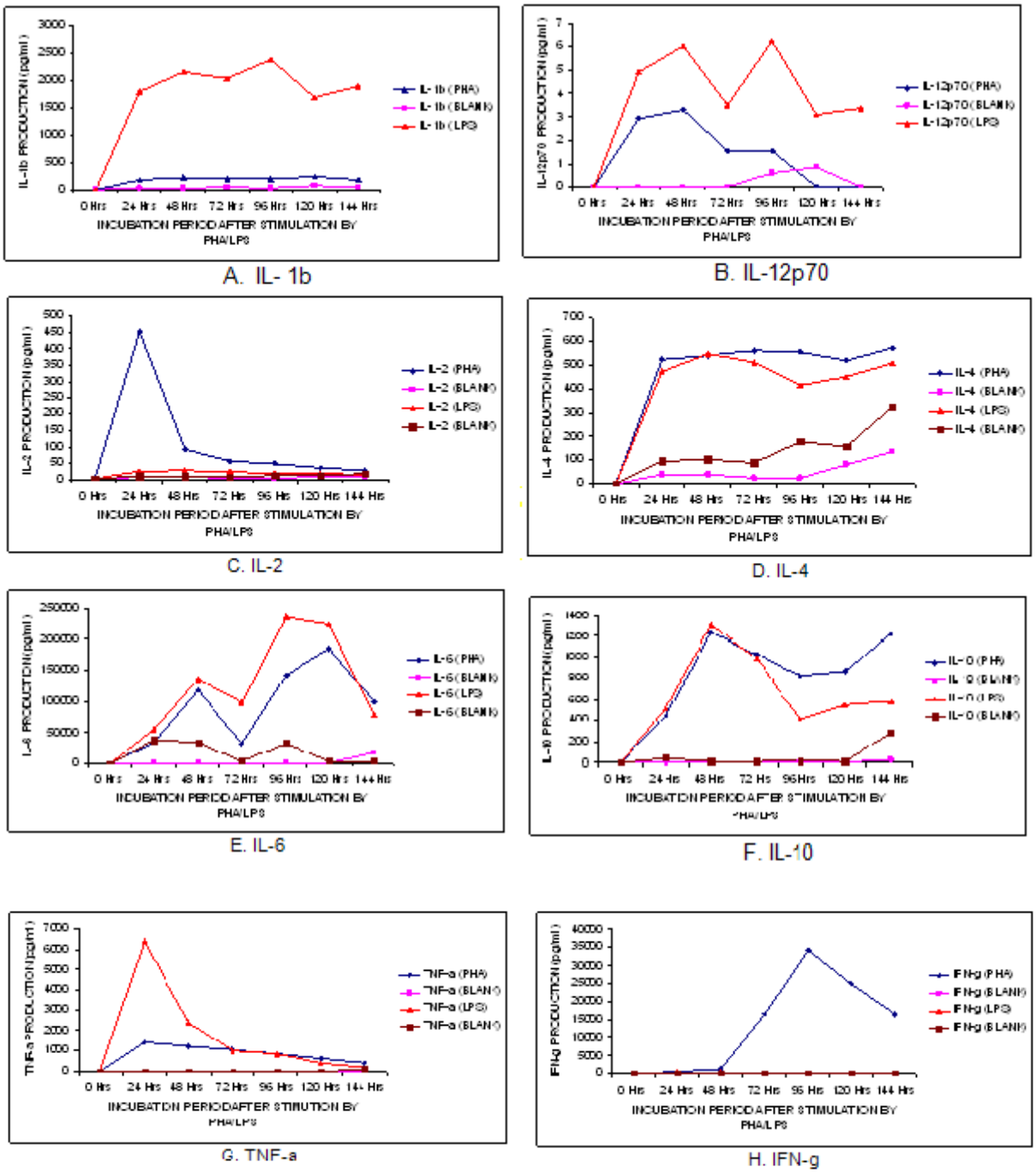

FIGURE 2

TIME KINETICS FOR GENERATION OF CYTOKINE EMPLOYING PHYTOHAEMAGGLUTININ (PHA) AND LIPPOPOLYSACCHARIDE (LPS) AS STIMULANT

DISCUSSION

Cytokines are involved in multiple aspects of physiology and pathology and are being used clinically.[19] The assessment of patients with various immunodeficiencies is likely to require information about their ability to produce cytokines. In view of the increasing awareness of the key role of cytokines in a variety of human diseases, assays for cytokine quantification have become a desirable one.[20, 21,22] The culture of whole blood approximates the state of circulating cells in vivo and contains physiological concentrations of factors that influence the immune cell function more closely than the culture of peripheral blood mononuclear cells (PBMC). Whole blood culture may be the most appropriate milieu in which to study ex vivo cell activation and cytokine production in vitro.[11]

CONCLUSION

The present study on kinetics (in term of time & dose) of cytokine production by stimulatory effects of PHA-L and LPS may lead to better understanding of disease and its management

REFERENCES

1. Kuby, 1994: Cytokines. *Immunology 2nd Ed. 297*
2. Iain, 2007: Role of cytokines in immune system. *Up to Date15.3*
3. O' Garra A, Murphy K 1994: *Curr Opin Immunol. Jun;6(3):458-66*
4. Raffi, 1995: Cytokines, HIV and the immune system. *AIDS Treatment Update Sep.*
5. Roitt Ivan M ,1996: The cytokine network. *Immunology 4th Ed 9.11*
6. Vilcek, J., Feldmann, M. 2004. Historical review: cytokines as therapeutics and targets of therapeutics. *Trends Pharmacol. Sci.* 25:201-209.
7. Oppenheim, J.J. 2001. Cytokines: past, present, and future. *Int. J. Hematol.* 74: 3-8.
8. Dixon MF and Philips Q. Cytokines . In Aids to Pathology. 4th edition. Churchill Livingstone,1993; 9 – 14.
9. Joseph L, Fink LM, Hauer jensen M. Cytokines in coagulation and thrombosis: a pre-clinical and clinical review. Blood, Coagul Fibrinolysis, 2002; 13(2): 105 – 116
10. Cytokines and interventional immunology David A. Hafler Nature Reviews Immunology 7, 423 (June 2007)
11. Murphy, K.; Travers, P.; Walport, M. (2007). "Signaling Through Immune System Receptors.". *Janeway's Immunobiology.* (7th ed.). London: Garland. ISBN 0815341237.
12. Borish, L., and L. Rosenwasser. 1996. Update on cytokines. J. Allergy Clin. Immunol. 97:719–730.
13. ROHIT K. KATIAL,1* DORIS SACHANANDANI,2 CAROLYN PINNEY, 2 AND MICHAEL M. LIEBERMAN2† "Cytokine Production in Cell Culture by Peripheral Blood Mononuclear Cells from Immunocompetent Hosts" CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, Jan. 1998, p. 78–81 Vol. 5, No. 1
14. Friberg, D., J. Bryant, W. Shannon, and T. Whiteside. 1994. In vitro cytokine production by normal human peripheral blood mononuclear cells as a measure of immuno competence or the state of

activation. Clin. Diagn. Lab. Immunol. 1:261–268.

15. Akira, S., T. Hirano, T. Taga, and T. Kishimoto. 1990. Biology of the multifunctional cytokines: IL6 and related molecules (ILl and TNF). FASEB J. 4:2860-2867.

16. Balkwill, F. R., and F. Burke. 1989. The cytokine network. Immunol. Today 10:299-304.

17. Kantor, T. V., D. F. Friberg, Jr., T. A. Medsger, R. B. Buckingham, and T. L. Whiteside. 1992. Cytokine production and serum levels in systemic sclerosis. Clin. Immunol. Immunopathol. 65:278-285.

18. Waage, A., P. Brandtzaeg, A. Halsteusen, P. Kierulf, and T. Esperile. 1989. The complex pattern of cytokines in serum from patients with meningococcal septic shock. J. Exp. Med. 169:333- 338.

19. Borden, E. C., and P. M. Sondell. 1990. Lymphokines and cytokines in cancer treatment. Immunotherapy realized. Cancer 65: 800-814.

20. Conlon, P. J. 1993. A rapid biologic assay for the detection of interleukin 1. J. Immunol. 131:1280-1287.

21. Gaffney, E. V. 1989. Interleukin 1: clinical significance and current assay protocols. Clin. Biotechnol. 1:5-11.

22. Gillis, S., M. M. Ferm, W. Ou, and K. A. Smith. 1978. T cell growth factor: parameters of production and quantitative microassay for activity. J. Immunol. 120:2027-2032.