Vol. 181, No. 1, 1991
November 27, 1991

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
Pages 179-183

INDUCTION OF MEMBERS OF THE IL-8/NAP-1 GENE FAMILY IN HUMAN T LYMPHOCYTES IS SUPPRESSED BY CYCLOSPORIN A

P.F. Zipfel, A. Bialonski and C. Skerka

Department of Molecular Biology, Bernhard Nocht Institute for Tropical Medicine, 2000 Hamburg 36, Germany

Received September 19, 1991

Human members of a family of structurally related cytokines, which play a role as effectors of inflammation, were analyzed for their expression and regulation in T lymphocytes. Members of this gene family include Platelet Basic Protein (PBP); Platelet Factor 4 (PF-4); IL-8/NAP-1; IP-10, a gamma interferon induced protein; GRO; pAT 464 and pAT 744. In resting T lymphocytes the RNAs of the individual genes could not be detected, but all genes were induced upon stimulation with PHA or with PHA/PMA. The induction of five genes was blocked by the immunosuppresive drug cyclosporin A (CSA), which appears to affect initial events in T cell activation. This expression in T lymphocytes, especially the sensitivity to CSA, indicates a common immunmodulatory role of these structural related proteins.

T lymphocytes regulate inflammatory processes by synthesis and secretion of a number of stimulatory molecules including C5a (1), Platelet activating factor (2) and leukotriene B4 (3). In addition to these well characterized chemotaxins, a number of novel polypeptides with chemotactic activity for cells participating in inflammatory processes have recently been isolated. Several factors or predicted proteins comprise the IL-8/NAP-1 gene family of structurally related molecules (for review see 4-6). The human members include: Platelet Basic Protein (PBP), which when processed forms ß-thromboglobulin (ß-TG), Connective Tissue Activating Peptide (CTAP-III) and Neutrophil Activating Peptide-2 (NAP-2) (7,8); Platelet Factor-4 (PF-4) (9); Interleukin-8 or Neutrophil Activating Peptide-1 (IL-8/NAP-1) (5); IP-10, a IFN-τ induced protein (10); GRO, a gene whose RNA is upregulated in transformed fibroblasts (11,12); pAT 464 and pAT 744, two genes which are induced in T lymphocytes (13). Individual members of this family of cytokines/lymphokines display diverse biological effects associated with inflammation, regulation of cell growth and differentiation, as well as control of the immune response (for review see 4-6).

While the individual genes of the IL-8/NAP-1 family are expressed in a variety of cells including fibroblasts and monocytes, their expression in T lymphocytes has not been studied in detail. In T cells the immunosuppressive agent CSA, has been shown to selectively inhibit the transcriptional activation of lymphokine genes and genes important for lymphocyte function (14-17).

Abbreviations: PBP - Platelet basic protein, ß-TG - ß-thromboglobulin, CSA - Cyclosporin A, CTAP-III - connective tissue activating peptide III, PF-4 - Platelet factor 4, NAP - neutrophil activating peptide, IP-10 - τ-interferon induced protein.

0006-291X/91 $1.50

To obtain further information of the immunmodulatory role of the individual members of the IL-8/NAP-1 gene family, we analyzed their expression and regulation in T lymphocytes. Here we show that the mRNAs of seven genes were induced upon activation of T cells and that the induction of five genes could be blocked by the immunosuppressive drug CSA.

Material and Methods

T cell isolation. Human peripheral blood T cells were obtained from buffy coats of normal donors (Blood Bank of the University Hospital Hamburg-Eppendorf) and were isolated as described (13). Briefly, after Ficoll-Hypaque centrifugation, T cells were enriched on nylon wool columns and were resuspended in RPMI medium supplemented with 10% heat inactivated FCS at a concentration of $2x10^6$ cells/ml.

Cell culture and stimulation. Jurkat cells were cultivated in RPMI, supplemented with 10% heat inactivated FCS, penicillin, streptomycin and gentamicin in humidified atmosphere at 37^0 C. They were stimulated with 1 ug/ml Phytohemagglutinin (PHA, Burroughs Wellcome, Research Triangle Park, NC), 25 ng/ml Phorbol 12-myristate 13-acetate (PMA, Sigma) or a combination of both in the presence or absence of 10 ug/ml cycloheximide (Sigma). Peripheral blood T lymphocytes were stimulated either with 1 ug/ml PHA and 25 ng/ml PMA or a combination of both. Cyclosporin A was used at a concentration of 1 ug/ml.

RNA isolation and Northern blotting. Total cellular RNA was extracted with guanidine thiocyanate, and isolated by centrifugation over CsCl (18). The RNA concentration was determined spectrophotometrically and 8 ug of total cellular RNA was separated by electrophoresis in a formaldehyde agarose gel and subsequently transferred to a nylon membrane (PALL membrane).

Labeling and hybridization. The cDNA probes were kindly supplied by K. Clementson (PBP), M. Poncz (PF-4), C. Weissmann (3-10C, i.e. IL-8/NAP-1), J. Ravetch (IP-10), and R. Sager (GRO). Plasmids were grown and isolated according to standard procedures (19). The cDNA inserts were excised, purified on low melt agarose gels and labeled with [^{32}P] by random priming (Amersham). After hybridization (5x Denhard's, 5x SSC, 0.1% SDS, 250 ug/ml denatured salmon sperm DNA, and 50% formamide) at 42^0 C the filters were washed at a final stringency in 0.1% SSC at 55^0 C.

Results

Expression of members of the IL-8/NAP-1 gene family in human peripheral blood T cells. T lymphocytes were stimulated with PHA, PMA or a combination of both, and steady state mRNA levels were analyzed by Northern blotting (Fig. 1). None of the genes analyzed was expressed in unstimulated, resting cells, but all were induced upon stimulation. However, two patterns of regulation were observed. The first group represented by PBP and PF-4 showed a weak response to stimulation. After 14 h mRNA encoding PBP was expressed by PHA-treatment, while PF-4 mRNA was also expressed by a combination of PHA and PMA. The second group of genes represented by IL-8/NAP-1, IP-10, GRO, pAT 464 and pAT 744, was induced by PHA and by a combination of PHA and PMA. The RNA of these genes was expressed for a prolonged time, and was still detectable 60 h after stimulation. In an early phase of activation PMA treatment did not result in mRNA induction, however, this agent caused mRNA expression upon longer stimulation. The regulation of the second group of genes resembles that of other lymphokine genes, such as the IL-2- or IFN-τ gene.

Regulation by Cyclosporin A. Due to their IL-2 like regulation in peripheral blood T cells and due to their role as cytokines/lymphokines, we asked

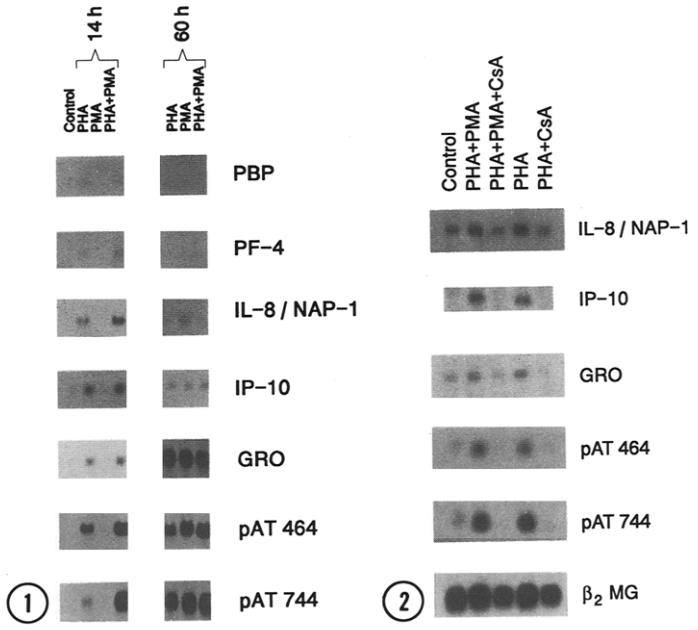

Figure 1. Expression of members of the IL-8/NAP-1 gene family in human peripheral blood T cells.
Total cellular RNA was isolated either from unstimulated T cells (control), or cells stimulated for the indicated time with PHA (1 µg/ml), PMA (25 ng/ml) or a combination of both. Equal amounts of RNA (8 µg) were loaded as confirmed by hybridizing the filters with a ß-2 microglobulin probe (data not shown). The apparent mRNA sizes as determined by comparison to the mobility of the 28S and 18S ribosomal RNAs were PF-4: 700 bp, PBP: 850 bp, IL-8/NAP-1: 1800 bp, IP-10: 1400, GRO: 1200 bp, pAT 464: 900 bp and pAT 744: 850 bp.

Figure 2. Effect of CSA on the expression of novel cytokines in T lymphocytes.
Total cellular RNA was isolated from unstimulated T lymphocytes (control) or from cells stimulated for 12 h with PHA (1 µg/ml) or a combination of PHA and PMA (25 ng/ml) in the presence or absence of CSA (1 µg/ml). Equal amounts of RNA (8 µg) were loaded as confirmed by rehybridizing the filters with a ß-2 microglobulin probe.

whether CSA could suppress the induction of the second group of genes. Stimulating T cells for 12 h either with PHA or a combination of PHA/PMA in the presence or absence of CSA showed that CSA blocked the induction of all five genes analyzed (Fig. 2). This regulation by CsA is in agreement with an immunmodulatory role of the encoded proteins. In this particular experiment a fraction of the cells was preactivated, as IL-8/NAP-1 mRNA could be detected even in unstimulated cells.

Expression of IL-8/NAP-1 in Jurkat cells. For the IL-8/NAP-1 mRNA we wanted to demonstrate its expression in T cells unambiguously. To this end the regulation was analyzed in the $CD4^+$ human helper T cell line Jurkat. As shown in Figure 3 IL-8/NAP-1 mRNA was not expressed in unstimulated, continuously proliferating cells, but could be induced by a combination of PHA and PMA. This induction was temporal, and stimulation in the presence of the protein biosynthesis inhibitor cycloheximide resulted in an increase of mRNA levels.

Discussion

We show that individual members (PBP, PF-4, IL-8/NAP-1, IP-10, GRO, pAT 464 and pAT 744) of the IL-8/NAP-1 gene family, require stimulation for their

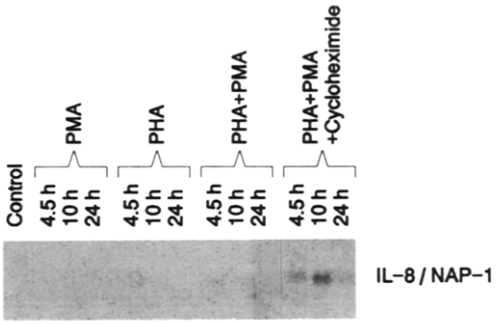

Figure 3. Expression of IL-8/NAP-1 mRNA in the human helper T cell line Jurkat. Total cellular RNA was isolated from untreated cells (control) or from cells stimulated with either PMA (25 ng/ml), PHA (1 µg/ml) or a combination of both. Cycloheximide was used at a concentration of 10 µg/ml.

induction in human T lymphocytes. None of the genes analyzed was detectably expressed in resting peripheral blood T cells, but their mRNAs were induced upon stimulation with PHA or PHA/PMA. In addition the induction of five genes could be blocked by the immunosuppressive drug CSA.

With the stimuli used mRNAs encoding PBP and PF-4 were found weakly induced in peripheral blood T cells. However, this low level of PBP and PF-4 induction is not due to stimulation of contaminating cells, as: (i) both genes are induced by PHA, which is thought to mediate signals through the T cell receptor complex molecules, and consequently acts as a T cell specific stimulus (20); and (ii) the T cells are highly enriched and contain over 95% $CD3^+$ cells. Therefore we conclude, that the mRNA encoding PBP and PF-4 can be induced in T lymphocytes by PHA, but that additional or other stimuli are required for optimal transcription.

Individual members of the IL-8/NAP-1 gene family are differently expressed in cells associated with inflammation and wound healing such as fibroblasts, endothelial cells and leukocytes. The coordinate induction of these genes and their lymphokine-like kinetics in peripheral blood T cells demonstrates a substantial role of these cells in the regulation of inflammatory events. Dispite this coordinate regulation in peripheral blood T lymphocytes, only a fraction of the genes analyzed displayed lymphokine-like regulation in the human $CD4^+$ helper T cell line Jurkat. Upon stimulation of Jurkat cells, which serve as a model system to study lymphokine regulation IL-8/NAP-1, pAT 464 and pAT 744 RNAs were expressed (Fig. 3, and 13). While the mRNAs encoding pAT 464 and pAT 744 were regulated similar to the IL-2 mRNA, the IL-8/NAP-1 mRNA was induced in the presence of the proteinbiosynthesis inhibitor cycloheximide. The remaining genes, which were expressed in peripheral blood T cells, but whose mRNA was not detected in Jurkat cells (data not shown), may be expressed in other cells, such as $CD8^+$ T cells.

CSA has been shown to inhibit T cell functions, especially the transcriptional induction of several lymphokine genes. The suppression by CSA observed for five genes of the IL-8/NAP-1 family is in agreement with an immunmodulatory role of their encoded proteins. However, the immunosuppressive

effects of CSA seems specific for T cells, as this drug failed to block the induction of pAT 464- and pAT 744 mRNA in monocytic cells (Data not shown). Although individual members of this family of related cytokines/lymphokines are expressed in a variety of different cells, in human peripheral blood T lymphocytes the genes are subject to T-cell specific regulation.

Acknowledgments

We thank Drs. Guido Trenn and Kim Henkle for a critical review of the manuscript and Dr. D. Römer, Sandoz Ltd. for providing Cyclosporin A.

References

1. Fernandez, H. N., Henson, P. M., Otani, A., and Hugli, T. E. (1978) J. Immunol. 120, 109-115.
2. Dewald, B., and Baggiolini, M. (1986) Biochem. Biophys. Acta. 888, 42-48.
3. Samuelson, B. (1983) Science 220, 568-575.
4. Wolpe, S.D., and Cerami, A. (1989) FASEB J. 3, 2565-2573.
5. Sherry, B., and Cerami, A. (1991) Curr. Opinion Immunol. 3, 56-60.
6. Baggiolini, M., Walz, A., and Kunkel, S.L. (1989) J. Clin. Invest. 84, 1045-1049.
7. Holt. J.C., Harris, M.E., Holt, A.M., Lange, E., Henschen, A., and Niewarowski, S. (1986) Biochem. 25, 1988-1996.
8. Walz, A., and Baggiolini, M. (1989) Biochem. Biophys. Res. Commun. 159, 969-975.
9. Deuel, T.F., Keim, P.S., Farmer, M., and Heinrikson, R.L. (1977) Proc. Natl. Acad. Sci. USA 74, 2256-2258.
10. Luster, A.D., Unkeless, J.C., and Ravetch, J.V. (1985) Nature 315, 672-676.
11. Anisowicz, A., Bardwell, L., and Sager, R. (1987) Proc. Natl. Acad. Sci. USA 84, 7188-7192.
12. Richmond, A., Balentien, E., Thomas, H.G., Flags, G., Barton, D.E., Spiess, J., Bordoni, R., Franke, U., and Dernyk, R. (1988) EMBO J. 7, 2025-2033.
13. Zipfel, P.F., Balke, J., Irving, S.G., Kelly, K., and Siebenlist, U. (1989) J. Immunol. 142, 1582-1590.
14. Emmel, E.A., Verweeij, C.L., Durand, D.D., Higgins, K.M., Lacy, E. and Crabtree, G.R. (1989) Science 246, 1617-1620.
15. Granelli-Piperno, A., Andrus, L., and Steinman, R.M. (1986) J. Exp. Med. 163, 922-937.
16. Nguyen, D.T., Eskandari, M.K., DeForge, L.E., Raiford, C.L., Strieter, R.M., Kunkel, S.L., and Remick, D.G. (1990) J. Immunol. 144, 3822-3828.
17. Liu, C. C., Rafii, S., Granelli-Piperno, A., Trapani, J.A., and Young, J.D.E. (1989) J. Exp. Med. 170, 2105-2118.
18. Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J., and Rutter, W.J. (1979) Biochemistry 18, 5294-5299.
19. Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989) Molecular cloning. Cold Spring Harbor Laboratory Press. Cold Spring Harbor, NY.
20. Weiss, A., and Stobo, J.D. (1984) J. Exp. Med. 160, 1284-1299.