Peptide-directed Suppression of a Pro-inflammatory Cytokine Response*

Received for publication, February 4, 2000, and in revised form, March 26, 2000
Published, JBC Papers in Press, April 5, 2000, DOI 10.1074/jbc.C000083200

Xue Yan Liu‡, Daniel Robinson‡, Ruth Ann Veach‡, Danya Liu‡, Sheila Timmons‡, Robert D. Collins§, and Jacek Hawiger¶

From the Departments of ‡Microbiology and Immunology and §Pathology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232-2363

Signal-dependent nuclear translocation of transcription factor nuclear factor κB (NF-κB) is required for the activation of downstream target genes encoding the mediators of immune and inflammatory responses. To inhibit this inducible signaling to the nucleus, we designed a cyclic peptide (cSN50) containing a cell-permeable motive and a cyclized form of the nuclear localization sequence for the p50-NF-κB1 subunit of NF-κB. When delivered into cultured macrophages treated with the pro-inflammatory agonist lipopolysaccharide, cSN50 was a more efficient inhibitor of NF-κB nuclear import than its linear analog. When delivered into mice challenged with lipopolysaccharide, cSN50 potently blocked the production of proinflammatory cytokines (tumor necrosis factor α and interferon γ) and significantly reduced the lethality associated with ensuing endotoxic shock. Based on specificity studies conducted with a mutated form of cSN50, a functional nuclear localization motif is required for this protective effect. Taken together, our findings demonstrate effective targeting of a cell-permeable peptide that attenuates cytokine signaling in vivo. This new class of biological response modifiers may be applicable to the control of systemic inflammatory reactions.

* This work was supported in part by USPHS National Institutes of Health Grant R01 DK54072. The use of core facilities in this study was supported in part by USPHS National Institutes of Health Grant R01 DK54072. This article must therefore be hereby marked "in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: Vanderbilt University Medical Center, MCN 5326, Nashville, TN 37232. Tel.: 615-343-8280; Fax: 615-343-8278; E-mail: jacek.hawiger@mcmail.vanderbilt.edu

‡ The abbreviations used are: TNFs, tumor necrosis factor α; LPS, lipopolysaccharide; NLS, nuclear localization sequence; NF-κB, nuclear factor κB; IFNγ, interferon γ; AP-1, activator protein 1; NFAT, nuclear factor of activated T cells; SRTFs, stress-responsive transcription factors; IL, interleukin; COX 2, cyclooxygenase 2; EM, minimal essential medium; FACS, fluorescence-activated cell sorting; ELISA, enzyme-linked immunosorbent assay; FITC, fluorescein isothiocyanate; PBS, phosphate-buffered saline; cSN50, cyclic SN50.

(SRTFs). As exemplified by NF-κB and NFAT, SRTFs are sequestered in the cytoplasm and mobilized to the nucleus following cellular stimulation (3–6). In turn, SRTFs interact with specific cognate sites present in the promoter region of genes encoding mediators of inflammatory and immune responses. For example, the gene encoding TNFα is regulated by NF-κB, NFAT, and ATF2/Jun, whereas the gene encoding IFNγ is controlled by NF-κB and NFAT (7–9).

Cytokine-mediated systemic inflammatory response can be evoked by lipopolysaccharide (LPS) (10–13). In response to LPS, monocytes, macrophages, and endothelial cells deploy SRTFs to their nuclear compartments (14–18). Persistent nuclear translocation of NF-κB in human and mouse mononuclear phagocytic cells correlates with the lethal outcome of systemic inflammatory response syndrome (19). Thus, development of this syndrome involves a multiplex process that is triggered by mobilization of NF-κB and other SRTFs to their nuclear sites of action and by systemic expression of proinflammatory cytokine mediators.

TNFα and IFNγ play a key role in the systemic inflammatory response syndrome induced by LPS, which has lethal effects in experimental animals. In keeping with this concept, animals deficient in receptors for these cytokines are resistant to lethal endotoxic shock (11, 20, 21). Thus, controlling the expression of these cytokine genes could provide an opportunity to ameliorate the ensuing systemic inflammatory responses. Considering the regulatory linkage between SRTFs and cytokine gene expression, we developed a method for noninvasive, intracellular delivery of peptides that interfere with SRTF signaling (for review see Ref. 22). The cell-permeable SN50 peptide we designed for these studies carries the hydrophobic region (h region) of the signal peptide as a membrane translocating motif and a nuclear localization sequence (NLS) derived from the p50-NF-κB1 subunit of transcription factor NF-κB. This linear peptide inhibits the nuclear import of NF-κB in human mononuclear cells and murine endothelial cells stimulated with the proinflammatory agonists LPS and TNFα (23). The SN50 peptide also inhibits the inducible nuclear import of AP-1, NFAT, and signal transducers and activators of transcription 1 (24, 25).

In terms of its mechanism of action, we have demonstrated in prior studies that the SN50 peptide interacts in vitro with a cytoplasmic NLS receptor comprised of the Rch1/importin (karyopherin)-β heterodimer (24). Peptide-directed interference with nuclear import of SRTFs in cultured cells attenuates the inducible expression of cyclooxygenase 2 (COX 2) protein (26) and the level of mRNA transcripts of the IL-2 and Fas ligand genes (24, 27). These data indicate that SN50 affects downstream SRTF-regulated genes. In this report, we demonstrate that in vivo delivery of a more potent cyclic analog
Peptide-directed Suppression of Cytokine Response

TABLE I

Sequences of cSN50, SN50, and SM peptides

| Peptide            | Cell-permeable sequence | Peptide-directed Suppression of Cytokine Response |
|--------------------|-------------------------|--------------------------------------------------|
| cSN50 peptide     | AAVALLPVAILLAPCYYQRRKLMPC (FITC) GGGAAVALLPVAILLAPCYYQRRKLMPC | Analysis of the cytokine concentrations by the standard according to the manufacturer's instruction. |
| FITC-cSN50 peptide| AAVALLPVAILLAPCYYQRRKLMPC                                         |                                                   |
| SN50 peptide      | AAVALLPVAILLAPCYYQRRKLMPC                                         |                                                   |
| SM peptide        | AAVALLPVAILLAPCYYQRRKLMPC                                         |                                                   |

Sequences are given in single letter amino acid code. Cell membrane-permeable sequence (underlined) is derived from the hydrophobic (h) domain of the Kaposis fibroblast growth factor signal sequence, and NLS is derived from NF-kB1 (23). Peptides were synthesized and purified as described previously (23, 24).

(cSN50) of this cell-permeable inhibitor of inducible nuclear import of NF-kB and other SKTFs suppresses systemic expression of proinflammatory cytokine genes and reduces LPS lethality.

**EXPERIMENTAL PROCEDURES**

**Peptide Synthesis**—The SN50 and SM peptides were synthesized, purified, filter-sterilized, and analyzed as described elsewhere (23–25). The SN50 peptide carries an NLS derived from NF-kB1 (p50), whereas the SM peptide functional motifs contains NLS motifs that preclude recognition by the NLS receptor, importin α (also called karyopherin α or R & D Systems, Minneapolis, MN). Serial dilutions were made to determine the cytokine concentrations by the standard according to the manufacturer's instruction.

**Murine Model of LPS-induced Lethal Shock**—Female C57BL/6 mice (The Jackson Laboratory, Bar Harbor, ME) that were 8–12 weeks old (20 g weight) were injected intraperitoneally with LPS (200 microliters, 4 mg/ml) from *E. coli* 0127:B8 (Difco). Cell-permeable cSN50, SN50 and SM peptides, or 0.8% pyrogen-free saline (diluent) were injected intraperitoneally before (30 min) and after (30, 90, 150, and 210 min and 6 and 12 h) LPS challenge. In experiments presented in Fig. 4D, the cSN50 peptide was not injected before LPS. Injections followed LPS challenge at 30, 90, 150, and 210 min and 6, 12, and 24 h. All injected agents were sterile and prepared in pyrogen-free saline. Animals were observed at 2-h intervals during the first 8 h, at 4-h intervals during the subsequent 16 h, and twice daily thereafter. Autopsies were performed shortly after death. Surviving animals were observed for 3 days in experimental groups or for 10 days in the groups shown in Fig. 4, B and D, after which they were sacrificed and autopsied. Formalin-fixed, paraffin-embedded sections of the liver, spleen, lungs, and kidneys were stained with hematoxylin and eosin to assess overall histology. Animal handling and experimental procedures were performed in accordance with the American Association for Accreditation of Laboratory Animal Care guidelines and approved by the Institutional Animal Care Committee.

**RESULTS**

**Inhibition of NF-kB by Linear and Cyclic SN50 Peptides in Cultured Murine Macrophages**—A cell-permeable cyclic peptide termed cSN50 was designed by inserting two cysteines flanking the NLS motif of the p50-NF-kB1 to form an intrachain disulfide bond (Table I). In addition, FITC-conjugated cSN50 peptide (FITC-cSN50) was synthesized to monitor its intracellular localization in cultured murine cells *in vitro* and in blood and spleen cells *in vivo*. As shown in Fig. 1A, fluorescence confocal laser scanning microscopy of cultured murine macrophage RAW 264.7 macrophages demonstrated intracellular accumulation of the FITC-cSN50 peptide. This finding is concordant with prior studies of its linear analog, SN50 (23). The cytoplasmic localization of FITC-cSN50 peptide is consistent with the intracellular distribution of its target, importin α (karyopherin α), in non-stimulated cells (24). When intracellular persistence of cSN50 and SN50 peptides was analyzed using indirect fluorescence (see “Experimental Procedures”) no detectable differences were observed (data not shown).

We next assessed whether the cSN50 peptide inhibits the nuclear import of NF-kB in cultured macrophage macrophages. Macrophages are known targets for the proinflammatory agonist, LPS, which can induce endotoxic shock. When tested in LPS-stimulated murine RAW264.7 macrophages, cSN50 inhibited the nuclear import of NF-kB at a concentration of 10 to 30 μM as compared with the 30–100-μM inhibitory range for SN50 peptide (Fig. 1B). During the same experiment the constitutively...
tively expressed nuclear CCAAT-binding factor, NF-Y, remained unchanged (results not shown). Based on quantitative phosphoimager analyses of these experiments \((n = 3)\) presented in Fig. 1C, cSN50 was 3 times more potent than the linear SN50 peptide \((IC_{50} = 7 \text{ versus } 22 \mu M)\). The cSN50 peptide was 3–10 times more inhibitory than the SN50 peptide toward the nuclear import of AP-1 and NFAT, respectively, when analyzed in Jurkat T cells stimulated with phorbol ester and ionomycin (results not shown). These experiments with the cultured cells provided a basis for the \emph{in vivo} delivery of cSN50 to examine its effect on the expression of cytokine genes and the outcome of LPS-induced lethal shock.

\textbf{Delivery of cSN50 Peptide to Blood and Spleen Cells in Vivo}—To determine whether cell-permeable cSN50 peptide injected intraperitoneally is delivered to blood cells and to the spleen, peripheral blood leukocytes and lymphocytes, as well as isolated spleen cells, were analyzed by FACS. As shown in Fig. 2, FACS analysis of the leukocyte/lymphocyte-rich fraction prepared from blood collected 20 min after intraperitoneal injection of FITC-cSN50 (0.7 mg) showed its transfer to circulating white blood cells based on a strong fluorescence signal detectable in examined cells (green tracing). Leukocytes and lymphocytes prepared from the blood of control mice injected with unconjugated FITC produced a background signal (red tracing) similar to that observed in leukocytes and lymphocytes isolated from the blood of animals receiving intraperitoneal saline injection (black tracing). Monitoring the delivery of FITC-cSN50 peptide to spleen cells, predominantly T and B lymphocytes, showed a strong fluorescence gain as compared with that measured in splenocytes obtained from mice injected with unconjugated FITC or saline (Fig. 2). FITC-cSN50 peptide was also detectable in other blood cell fractions, including red blood cells, analyzed by FACS (results not shown). There was no detectable hemolysis in blood samples collected from cell-permeable peptide-treated animals. These results indicate that FITC-cSN50 peptide injected intraperitoneally was rapidly delivered to circulating blood cells and to the spleen. This type of analysis cannot establish what fraction of intraperitoneally injected FITC-cSN50 peptide was delivered to blood and spleen cells.

\textbf{cSN50 Peptide Suppresses Cytokine Expression in Vivo}—Injection of LPS into humans, primates, or mice leads to an early burst of proinflammatory cytokine mediators of endotoxic shock such as TNF\(_\alpha\), IL-1, and IFN\(\gamma\) (29–35). To test whether \emph{in vivo} delivery of cSN50 will attenuate expression of cytokine genes regulated by NF-\(\kappa\)B and other SRTFs, we injected C57BL/6 mice intraperitoneally with 800 \(\mu\)g of LPS from \textit{E. coli} serotype 0127:B8 (LD\(_{100} = 800 \mu\)g). Consistent with prior studies (36), mice challenged with LPS showed an early burst of TNF\(\alpha\) expression at 1–2 h, followed by increased IFN\(\gamma\) production peaking at 24 h (Fig. 3). In contrast, there was no rise in TNF\(\alpha\) expression in the cSN50 peptide-treated mice (1.5 mg given intraperitoneally in 7 injections 30 min before to 12 h after LPS challenge). This effect was statistically significant \((p < 0.004)\) using the area under the curve method and the Student’s \(t\) test (37). Likewise, the more progressive rise of IFN\(\gamma\) in LPS-challenged mice was suppressed by cSN50 pep-
cSN50 peptide-treated and control mice was statistically significant \( (p < 0.04) \). Thus, in vivo delivery of cSN50 resulted in a striking attenuation of expression of genes encoding two key proinflammatory mediators of endotoxic shock, TNF\(\alpha\) and IFN\(\gamma\). This is consistent with prior in vitro data on SN50 peptide-induced attenuation of expression of genes encoding IL-2, Fas ligand, and COX 2 measured as mRNA transcripts or protein levels in cultured cells (24, 26, 27).

**In Vivo Delivery of cSN50 Peptide Prevents LPS-induced Lethal Shock**—Almost complete in vivo suppression of the key cytokine mediators of endotoxic shock by cSN50 correlated with a reduction in mouse lethality. As shown in Fig. 4A, all LPS-injected animals died within 72 h. In contrast, mice treated with cSN50 (1.5 mg given in 7 injections 30 min before LPS challenge) were essentially protected throughout the 72-h period, as evidenced by the cumulative survival rate of 90\% (Fig. 4B). Survivors observed for the subsequent 10 days showed no apparent signs of disease. This protective effect of cSN50 was reduced (50\% survival) when the dose was decreased to 0.7 mg per injection (Fig. 4C). To determine whether the cSN50 peptide reduces lethality when administered after exposure to LPS, the first peptide dose was given 30 min after endotoxin. The survival rate was 60\%, indicating that the cSN50 peptide attenuates endotoxic shock, if given shortly after exposure to LPS (Fig. 4D). The in vivo protective effect was lost if the functional NLS motif was mutated, as in the SM peptide. Mutations in the NLS motif preclude recognition by the NLS receptor, importin \( \kappa \) (also called karyopherin \( \alpha \) or Rch 1) (24). All SM peptide-treated mice died within 72 h (Fig. 4E).

Based on the log rank test (38), the difference in survival rate between cSN50 peptide-treated and control mice was statistically significant \( (p < 0.001) \). The cSN50 peptide appears to be at least 3 times more potent in vivo than its linear analog, SN50 (1.5 mg given in 7 injections 30 min before to 12 h after LPS challenge; results not shown). This is consistent with the differences in inhibitory potency in cultured cells (Fig. 1C). Cumulatively, in vivo data obtained with the cSN50 peptide indicate that this inhibitor of nuclear import of NF-\( \kappa \)B and other SRTFs is effective in attenuating systemic expression of proinflammatory cytokine mediators of endotoxic shock and its lethal outcome.

**DISCUSSION**

When delivered to cultured cells, the cell-permeable cyclic peptide, cSN50, inhibits inducible nuclear import of NF-\( \kappa \)B and other SRTFs. We have demonstrated not only in vitro delivery of cSN50 to murine macrophages but also in vivo targeting of this new cyclic peptide to blood and spleen cells in mice. Accordingly, we tested whether the in vivo delivery of cSN50 modifies the expression of proinflammatory cytokines and lethal shock in response to LPS. These in vivo experiments demonstrated that cSN50 attenuates expression of genes encoding two key mediators of endotoxic shock, TNF\(\alpha\) and IFN\(\gamma\), as well as provides a highly significant protection of mice from LPS-induced lethal shock. Remarkably, in vivo delivery of cSN50 at its optimal concentration resulted in a 90\% survival rate among mice receiving LD\(_{100}\) of LPS.

As expected from studies of SN50 (24), its cyclic analog, cSN50, inhibits nuclear import of NF-\( \kappa \)B and other SRTFs. However, cyclization of SN50 appears to enhance its inhibitory effects toward NF-\( \kappa \)B signaling. The enhanced inhibitory potency of cSN50 does not appear to be due to its longer intracellular persistence than that of SN50 peptide. Rather, it may reflect a higher affinity of cSN50 versus SN50 for importin \( \alpha \) (karyopherin \( \alpha \)), the intracellular target of these peptides (24). The cSN50 peptide fails to inhibit the constitutively expressed...
nuclear transcription factor, NF-Y, suggesting that the peptide preferentially affects inducible nuclear import. Based on prior studies with SN50 peptide, we postulate that its intracellular target is most likely the cytoplasmic NLS receptor, Rch 1 (importin α/karyopherin α) (24). The broad inhibitory range of cSN50 toward the nuclear import of NF-κB and other SRTFs is an advantage rather than a drawback to its in vivo delivery for the following reasons. First, the cytokine genes that mediate systemic inflammatory response are regulated by multiple SRTFs. In particular, the gene encoding TNFα is regulated by SRTFs. In particular, the gene encoding TNFα is regulated by SRTFs, cSN50 represents a new class of anti-inflammatory agents capable of suppressing systemic inflammatory responses in vivo.

In summary, these experiments indicate that cSN50 can be delivered in vivo to cells involved in synthesis of proinflammatory cytokines. In turn, the expression of genes encoding these key mediators of endotoxic shock is suppressed, resulting in reduced mortality. Although endogenous inhibitors of cytokine signaling, such as members of the CIS/SOCS/JAB/SSI family, negatively regulate signal transduction (39), all exogenous inhibitors of LPS toxicity tested in vivo and reported until now are targeted toward the interaction of LPS or cytokines with their cognate receptors (29–32, 34, 40, 41). In contrast, the cell-permeable peptides described here are targeted intracellularly (22). As a reversible inhibitor of the nuclear import of SRTFs, cSN50 represents a new class of anti-inflammatory agents capable of suppressing systemic inflammatory responses in vivo. In keeping with this concept, SN50 is protective when delivered to mice challenged with a low dose of LPS in combination with β-galactosamine, which renders mice hypersensitive to LPS (42). In addition, preliminary experiments indicate that in vivo delivery of the cSN50 peptide is effective in blocking lethal shock induced by the superantigen, staphylococcal enterotoxin B, that robustly activates a subset of T cells (data not shown).

Acknowledgments—We thank Dean Ballard for most helpful advice in preparation of this manuscript, William DuPont and Shiva Gautham for statistical consultations, Louis Sele for help with some in vivo experiments, David McFarland for FACS analysis, and Erica Holleran and Traci Tidwell for assistance in preparation of the manuscript.

REFERENCES

1. Crabtree, G. R., and Clibstone, N. A. (1984) Annu. Rev. Biochem. 63, 1045
2. Schindler, C., and Darnell, J. E., Jr. (1995) Annu. Rev. Biochem. 64, 621–651
3. Baldwin, A. S. (1996) Annu. Rev. Immunol. 14, 649–681
4. Gilmore, T. D. (1999) Oncogene 18, 6842–6844
5. Raa, O., Luo, C., and Hogan, P. G. (1997) Annu. Rev. Immunol. 15, 707–747
6. DiNayy, B. G., and Aggarwal, B. B. (1999) Annu. Rev. Med. 50, Suppl. 1, 12–113
7. Goldfeld, A. E., McCaffrey, P. G., Strominger, J. L., and Rao, A. (1993) J. Exp. Med. 176, 1365–1379
8. Tsai, E. Y., Yie, J., Thanos, D., and Goldfeld, A. E. (1996) Mol. Cell. Biol. 16, 5232–5244
9. Sica, A., Dormann, L., Viggiano, V., Cippitelli, M., Ghosh, P., Rice, N., and Young, H. A. (1997) J. Biol. Chem. 272, 50442–50442
10. Hawiger, J. (1994) in Endotoxin and the Lungs (Brigham, K. L., ed) Vol. 77, pp. 69–82, Marcel Dekker, Inc., New York
11. Car, B. D., Eng, V. M., Schnyder, B., Ozmen, L., Huang, S., Gallay, P., Heumann, D., Aguet, M., and Ryffel, B. (1994) J. Exp. Med. 179, 1437–1444
12. Mackman, N. (1995) FASEB J. 9, 883–889
13. Ulevitch, R. J., and Tobias, P. S. (1995) Annu. Rev. Immunol. 13, 437–457
14. Cordie, S. R., Donald, R., Read, M. A., and Hawiger, J. (1993) J. Biol. Chem. 268, 11803–11810
15. Donald, R., Ballard, D. W., and Hawiger, J. (1993) J. Biol. Chem. 270, 9–12
16. Mackman, N., Brand, K., and Edgington, T. S. (1991) J. Exp. Med. 174, 1517–1526
17. Pugin, J., Schurer-Maly, C. M., Leturcq, D., Morigiary, A., Ulevitch, R. J., and Tobias, P. S. (1999) Proc. Natl. Acad. Sci. U. S. A. 90, 2744–2748
18. Read, M. A., Cordie, S. R., Veach, R. A., Carlisle, C. D., and Hawiger, J. (1993) Proc. Natl. Acad. Sc. U. S. A. 90, 9887–9891
19. Bohrer, H., Qui, F., Zimmerman, T., Zhang, Y., Jlmer, T., Mannel, D., Dettbarger, B. W., Stern, D. M., Saeger, H. D., Ziegler, R., Bierhaus, A., Martin, E., and Nawrot, P. P. (1997) J. Clin. Invest. 100, 972–985
20. Rothé, J., Lesslerauer, W., Lottescher, H., Lang, Y., Kobel, P., Kostgen, F., Althage, A., Zinkernagel, R., Steinmetz, M., and Bluethmann, H. (1993) Nature 364, 798–802
21. Pfieffer, K., Matsuzaki, T., Kundig, T. M., Wakeham, A., Kishihara, K., Shahinian, A., Wiegmann, K., Ohashi, P. S., Kronke, M., and Tak, T. W. (1993) Cell 73, 457–467
22. Hawiger, J. (1999) Curr. Opin. Chem. Biol. 3, 89–94
23. Lin, Y. Z., Yao, S. Y., Veach, R. A., Torgersson, T. R., and Hawiger, J. (1995) J. Biol. Chem. 270, 14355–14358
24. Torgersson, T. R., Colosia, A. D., Donahue, J. P., Lin, Y. Z., and Hawiger, J. (1998) J. Biol. Chem. 273, 6984–6992
25. Zhang, L., Torgersson, T. R., Liu, Y. X., Timmons, S., Colosia, A. D., Hawiger, J., and Tam, J. P. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 9184–9189
26. Abatie, A., Obere, S., and Schröder, H. (1998) Prostaglandins Other Lipid Mediat. 56, 277–290
27. Kasibhatla, S., Genestier, L., and Green, D. R. (1999) J. Biol. Chem. 274, 987–992
28. Liu, X-Y., Timmons, S., Lin, Y-Z., and Hawiger, J. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 11819–11824
29. Tracey, K. J., Fong, Y., Hesse, D. G., Manogue, K. R., Lee, A. T., Kuo, G. C., Lowry, S. F., and Cerami, A. (1987) Nature 320, 662–664
30. Michie, H. R., Manogue, K. R., Spriggs, D. R., Revhaug, A., D’Oreyer, S., Dinaro, C. A., Cerami, A., Wolff, S. M., and Wilmore, D. W. (1988) N. Engl. J. Med. 318, 1481–1486
31. Richardson, R. P., Rhyme, C. M., Fong, Y., Hesse, D. G., Tracey, K. J., Marano, M. A., Lowry, S. F., Antonacci, A. C., and Calvano, S. E. (1989) Ann. Surg. 210, 239–245
32. Remick, D. G., Strierer, R. M., Ekedamri, K. M., Nguyen, D. T., Genord, M. A., Raiford, C. L., and Kunkel, S. L. (1999) Am. J. Pathol. 156, 49–60
33. van Deventer, S. J., Buller, H. R., ten Cate, J. W., Jarden, L. A., Hack, C. E., and Sturk, A. (1996) Blood 80, 2520–2526
34. Alexander, H. R., Doherty, G. M., Buresh, C. M., Venzon, D. J., and Norton, A. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 80–83
35. Kuhns, D. B., Alvord, W. G., and Gallin, J. I. (1995) J. Cell Biol. 136, 1437–1444
36. Dinarello, C. A. (1998) Annu. Rev. Immunol. 16, 457–499
37. Galanos, C., Freundgen, M. A., and Reutter, W. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 5939–5943

---

2 X. Y. Liu, D. Robinson, R. A. Veach, S. Timmons, R. D. Collins, and J. Hawiger, unpublished observations.
