Brief Definitive Report

C-reactive Protein: A Physiological Activator of Interleukin 6 Receptor Shedding

By Simon A. Jones,* Daniela Novick,§ Sankichi Horiuchi,‖ Naoki Yamamoto,‖ Alexander J. Szalai,‡ and Gerald M. Fuller*

*From the *Department of Cell Biology and the ‡Department of Medicine, Division of Clinical Immunology and Rheumatology, University of Alabama at Birmingham, Birmingham, Alabama 35294; the §Department of Molecular Genetics, Weizmann Institute of Science, Rehovot 76100, Israel; the ‖Department of Microbiology, Tokyo Medical and Dental University, Tokyo 113, Japan*

Summary

The soluble interleukin 6 receptor (sIL-6R) circulates at elevated levels in various diseases. This suggests that inflammatory mediators control sIL-6R release. Through examination of human neutrophils, it was found that the acute phase reactant C-reactive protein (CRP) activates a threefold increase in sIL-6R production. Maximal release occurred after 30–60 min exposure to CRP (50 μg/ml), and was mimicked by peptides corresponding to amino acid residues 174–185 and 201–206 of native CRP. A third peptide fragment (77–82) had no effect. Differential mRNA splicing did not account for the CRP-mediated release of sIL-6R, since this isoform was not detected in conditioned media. Furthermore, stimulation of neutrophils with CRP or with peptides 174–185 or 201–206 promoted a loss of membrane-bound IL-6R, suggesting release by proteolytic shedding. The metalloprotease inhibitor TAPI had only a marginal effect on CRP-mediated sIL-6R release, suggesting that shedding occurs via a mechanism distinct from that previously reported. It well established that IL-6 stimulates the acute phase expression of CRP. Our current findings demonstrate a novel relationship between these two mediators, since CRP may affect IL-6–mediated inflammatory events by enabling formation of the sIL-6R/IL-6 complex.

Key words: cytokines • interleukin 6 • soluble receptors • acute phase proteins • inflammation

The receptor complex that mediates the biological activities of IL-6 consists of two distinct membrane-bound glycoproteins, an 80-kD cognate receptor subunit (IL-6R, CD126) and a 130-kD signal transducing element (gp130) (1). Although IL-6R expression is confined to select cell types, IL-6 can activate cells lacking the cognate receptor via a soluble IL-6 receptor (sIL-6R) (2). Once bound to IL-6, the resulting sIL-6R/IL-6 complex acts as an agonist that is capable of activating cells through membrane-bound gp130. Since expression of gp130 is essentially ubiquitous, the sIL-6R/IL-6 complex has the potential to stimulate cell types that are not inherently responsive to IL-6 alone. Recent studies show that the sIL-6R/IL-6 complex can induce myocardial hypertrophy (3), cellular proliferation (4, 5), and osteoclast formation (6). In addition, the active complex has been reported to regulate leukocyte recruitment (7), and to promote the proinflammatory stimulation of endothelial cells (7, 8). These latter findings appear to contrast with the antiinflammatory properties assigned by some to IL-6 (9, 10), and suggest that the sIL-6R/IL-6 complex not only potentiates IL-6 signaling, but may also modify its biological activities.

Two distinct isoforms of sIL-6R have been identified. The first is shed from the cell surface via proteolytic cleavage of the membrane-bound IL-6R (PC–sIL-6R [11, 12]), whereas the second is secreted as the product of differential mRNA splicing (DS–sIL-6R [13, 14]). The sIL-6R is present in the plasma of healthy individuals (~25–35 ng/ml), and these levels are significantly elevated in diseases such as rheumatoid arthritis, multiple myeloma, and T cell abnormalities such as AIDS and adult T cell leukemia (15–17). The increased concentration of sIL-6R in these disease states suggests that some inflammatory event either stimulates release of PC–sIL-6R, or increases the expression of DS–sIL-6R. Since the inflammatory potential of IL-6 is modulated through binding the sIL-6R, identifying physiological mediators of sIL-6R generation is of central importance to understanding the significance of this soluble receptor in disease.

Bacterial pore-forming toxins (18) and FMLP (8) are known to activate generation of PC–sIL-6R, whereas oncostatin-M was recently shown to stimulate the release of DS–sIL-6R from a human hepatoma cell line (19). However, to date no endogenously produced activator of PC–

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/99/02/599/06 $2.00
Volume 189, Number 3, February 1, 1999 599–604
http://www.jem.org

sIL-6R shedding has been identified. In the present study, C-reactive protein (CRP), at concentrations likely to be encountered during an acute phase response, was found to provoke release of the membrane-bound IL-6R from human neutrophils. Thus, CRP has the potential to influence the inflammatory properties of IL-6 through facilitating formation of the sIL-6R/IL-6 complex.

Materials and Methods

Materials. Culture reagents were obtained from GIBCO BRL, and purified human CRP from Calbiochem-Novabiochem Corp. Peptides corresponding to CRP amino acid residues 77–82 (VGGSEI), 174–185 (IYLGGPFSPNVL), and 201–206 (KPQLWP) were from Sigma Chemical Co. Biotinylated anti–human IL-6R antibody (BAF-227) was from R&D Systems. Anti-DS–sIL-6R mAb (2F3) was generated as described previously (20). Dr. R.A. Black (Immunex Corp.) provided the TNF-α–protease inhibitor, TAPI. Lymphoprep was from Nycomed Pharma, and ImmunoPure 3,3′, 5,5′-tetramethylbenzidine (TMB) from Pierce Chemical Co.

Isolation of Human Neutrophils. Venous blood (20 ml) was obtained by antecubital venipuncture from nonsmoking healthy individuals (aged 26–54), mixed with an equal volume of 2% (wt/vol) dextran/0.8% (wt/vol) trisodium citrate in PBS (pH 7.4), and erythrocytes were allowed to sediment. Plasma was collected, underlayered with Lymphoprep (2:1 [vol/vol] plasma/Lymphoprep), and centrifuged at 4°C for 20 min at 800 *g*. The neutrophil-containing pellet was collected, and contaminating erythrocytes were removed by hypotonic lysis. Neutrophil preparations were found to be >95% pure as assessed by differential Wright staining. Before use, neutrophils were resuspended in serum-free RPMI 1640 containing 2 mM l-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin.

Determination of sIL-6R Production. Neutrophils (2×10^6 cells) were treated as described in the figure legends. Culture medium was harvested and stored at −80°C until required. Concentration of sIL-6R was determined using an ELISA procedure. Microtiter 96-well plates were coated with 10 μg/ml anti–human IL-6R mAb (mAb 17.1; reference 21) and blocked at 4°C with 0.5% BSA. sIL-6R standards and unknowns were added and incubated at room temperature for 2 h. To detect bound sIL-6R, biotinylated anti–human IL-6R antibody (50 ng/ml BAF-227) was added for 2 h at room temperature, followed by a 20-min incubation with horseradish peroxidase–conjugated streptavidin. Plates were washed between each step with PBS containing 0.1% Tween 20. Peroxidase activity was determined using TMB as a substrate. The reaction was stopped with 1.8 M H_2SO_4, and absorbance was measured at 450 nm. To detect DS–sIL-6R, the capture antibody was replaced with 20 μg/ml anti-DS–sIL-6R antibody (mAb 2F3), and ELISA was performed as described using baculovirus-expressed DS–sIL-6R as a standard (20). The lower limit of detection for sIL-6R and DS–sIL-6R was 10 and 50 pg/ml, respectively.

Flow Cytometry. Loss of IL-6R expression from the neutrophil cell surface after stimulation was monitored by cytofluorometry (FACScan®; Becton Dickinson) as described (20). Values are expressed as the percent reduction in mean fluorescence units (MFU) from nonstimulated control cells: $MFU = (FU_{experimental} - FU_{autofluorescence})/(FU_{control} - FU_{autofluorescence})$.

Statistical Analysis. Statistical analysis was performed using Student's *t* test incorporated into the SigmaPlot (version 2.01) graphics program. A $P < 0.05$ indicated a statistically significant difference.

Results

C-reactive Protein Stimulates Production of sIL-6R by Human Neutrophils. Examination of human neutrophils obtained from 10 independent donors showed that CRP activates sIL-6R production (Fig. 1). In each case, basal sIL-6R release was significantly increased ($P < 0.0001$) after exposure to 50 μg/ml CRP, with the extent of sIL-6R production ranging between 86 and 234 pg/ml after CRP stimulation compared with 21–84 pg/ml for controls. On average, CRP resulted in a 3.06 ± 1.03–fold induction of sIL-6R levels (Fig. 1 B). In contrast, activation of human neutrophils with IL-4 or IL-10 had no effect on sIL-6R generation (data not shown). As shown in Fig. 2 A, production of sIL-6R increased rapidly, with optimal release occurring between 30 and 60 min after CRP addition. Generation of sIL-6R was also dose-dependent, with 50 μg/ml CRP inducing a maximal response (Fig. 2 B). Release of the sIL-6R in response to a single exposure to CRP was transient, since levels returned to baseline within 4–5 h after stimulation. Addition of a second CRP dose 2 h after the initial CRP stimulation did not further enhance production (data not shown).

Peptides Derived from CRP Activate sIL-6R Production. Neutrophil stimulation has been shown to activate the cleavage of native CRP into biologically active peptide fragments

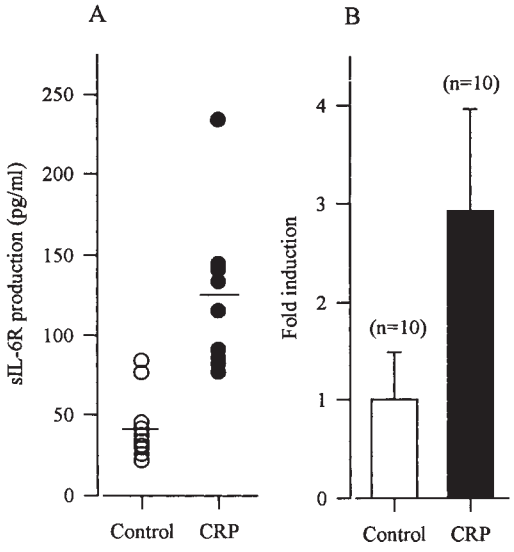

Figure 1. Production of sIL-6R by CRP-activated human neutrophils. (A) Human neutrophils (2×10^6 cells) from 10 independent donors were stimulated at 37°C, 5% CO_2 with 50 μg/ml CRP for 30 min, and the concentration of sIL-6R in culture medium was determined by ELISA. Horizontal bars represent the mean sIL-6R concentration for control (43 pg/ml) and CRP-activated (129 pg/ml) neutrophils. The CRP-induced release of sIL-6R was statistically significant ($P < 0.0001$) as determined by Student's *t* test. (B) The magnitude of CRP-induced sIL-6R production is shown for each donor. For controls, the mean concentration of 43 pg/ml was set to 1 and compared with the nonstimulated release for each donor. Values are expressed as mean fold induction ± SD ($n = 10$).

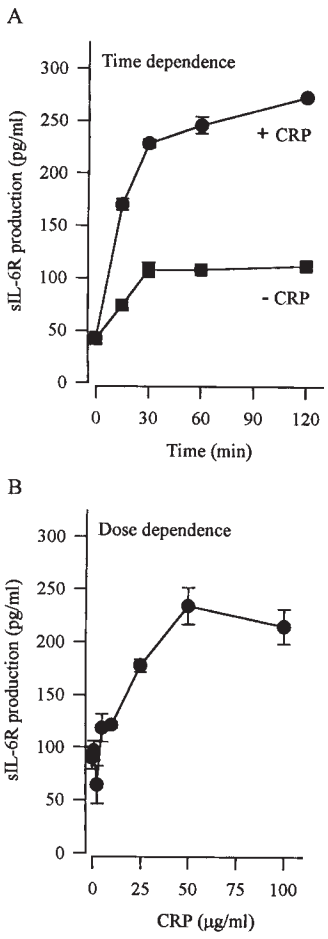

Figure 2. Dose- and time-dependent release of the sIL-6R in response to CRP. Neutrophils (2×10^6 cells) were stimulated at 37°C, 5% CO_2 with either (A) the indicated concentration of CRP for 45 min, or (B) for the indicated time period with (+CRP) and without (−CRP) 50 μg/ml CRP. After appropriate incubation, culture medium was harvested and sIL-6R concentrations were determined. Values represent the mean production ± SD ($n = 3$).

(22). In particular, peptides corresponding to amino acid residues 77–82, 174–185, and 201–206 profoundly influence neutrophil responses (23, 24). Accordingly, human neutrophils were incubated with each of these peptides and their capacity to augment sIL-6R production was determined. As shown in Fig. 3, (174–185)CRP and (201–206)CRP stimulated sIL-6R production in a dose-dependent manner, whereas peptide (77–82)CRP had little or no effect.

CRP-activated Release of sIL-6R Occurs via Loss of the Cognate IL-6R. The sIL-6R can be released through proteolytic shedding of the cognate IL-6R or secreted as the product of differential IL-6R mRNA splicing (11–14). Flow cytometry using neutrophils from three separate donors showed that a 30-min exposure to 100 μg/ml native CRP resulted in a 44 ± 2.5% loss of the cognate IL-6R. Similarly, 100 μg/ml (174–185)CRP and (201–206)CRP stimulated a 33 ± 6.2% and 24 ± 0.3% reduction in the surface expression of IL-6R, respectively, whereas peptide (77–82)CRP had little effect (data not shown). This indicates a role for CRP in the activation of IL-6R shedding. To verify this conclusion, the concentration of DS–sIL-6R was determined in conditioned media from CRP-activated neutrophils, using an antibody specific for the unique COOH-terminal amino acid sequence (GSRRRGSCGL) of DS–sIL-6R (20). As shown in Table I, no detectable level of DS–sIL-6R could be identified either before or after CRP stimulation. Interestingly, no correlation could be established between elevated systemic CRP concentrations and DS–sIL-6R levels in patients suffering from various clinical disorders (Horiuchi, S., and N. Yamamoto, unpublished data).

Partial Inhibition of sIL-6R Production by TAPI. Hydroxamic acid–based metalloprotease inhibitors such as TAPI are known to prevent shedding of various cell surface proteins (25–27), including the IL-6R (12, 20). Surprisingly, the CRP-induced release of sIL-6R by neutrophils was only partially blocked by TAPI (~20–25%; Fig. 4). Consistent with previous reports (12, 20), TAPI inhibited 70–75% of the phorbol ester–stimulated sIL-6R production by monocytic THP-1 cells (data not shown). Thus, the mechanism responsible for CRP-induced release of the cognate IL-6R from human neutrophils may be distinct from that described for monocytic cells.

Discussion

Elevated levels of the sIL-6R have been associated with the pathology of several disease states. This implies that production of the sIL-6R is increased as part of the inflam-

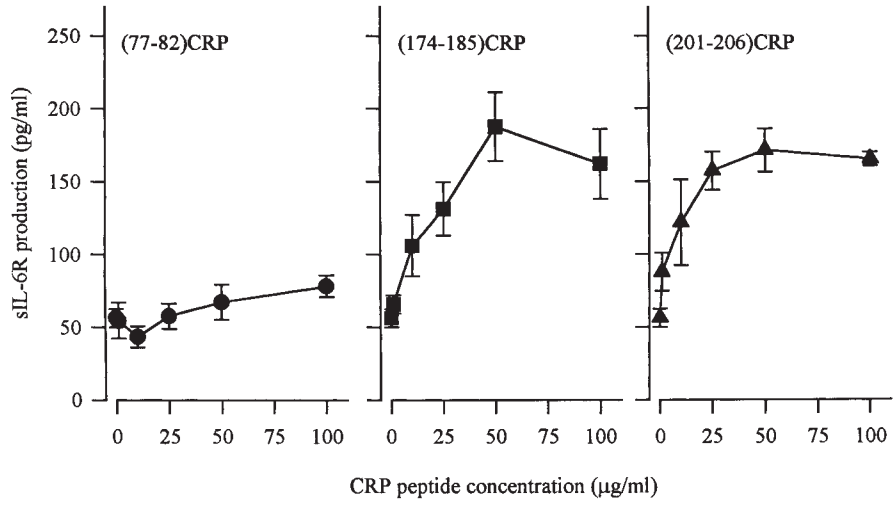

Figure 3. Peptides derived from CRP activate sIL-6R production by human neutrophils. Neutrophils (2×10^6 cells) were stimulated for 45 min at 37°C, 5% CO_2 with peptide (77–82)CRP, (174–185)CRP, or (201–206)CRP. sIL-6R concentrations are expressed as the mean ± SD ($n = 3$). Release of sIL-6R in response to 50 μg/ml CRP was 177.4 ± 6.5 pg/ml.

Table I. *sIL-6R Derived from Differential mRNA Splicing Does Not Account for the CRP-induced Release of sIL-6R*

CRP	DS–sIL-6R	Total sIL-6R
μg/ml	*pg/ml*	*pg/ml*
0	<LD	90.0 ± 10.6
1	<LD	96.8 ± 9.0*
10	<LD	118.5 ± 13.4‡
25	<LD	178.0 ± 5.9‡
50	<LD	234.0 ± 17.4‡

Neutrophils (2×10^6 cells) were stimulated with CRP for 45 min, and levels of total sIL-6R and DS–sIL-6R in conditioned medium were quantified (see Materials and Methods). Values represent the mean ± SD ($n = 3$). In the case of DS–sIL-6R, levels were below the limit of detection for the ELISA (<LD). *$P < 0.05$; ‡$P < 0.01$ vs. nonstimulated control.

matory response. However, little is known regarding the factors that might regulate sIL-6R generation. In this study, physiological concentrations of native CRP and biologically relevant CRP-derived peptides were found to stimulate sIL-6R production by human neutrophils. Release of this soluble receptor was rapidly induced after CRP treatment and occurred via shedding of the cognate IL-6R from the cell surface. C-reactive protein represents the first known endogenous activator of this process. The observation that release of sIL-6R is only partially prevented by the hydroxamic acid–based metalloprotease inhibitor TAPI is of particular interest, since IL-6R shedding in response to phorbol esters and ionomycin has been shown to be prevented by this agent (12, 20). Thus, in neutrophils, shedding of the IL-6R presumably occurs through a unique proteolytic mechanism. Indeed, under certain experimental conditions the phorbol ester–induced shedding of L-selectin (CD62L) from human neutrophils has also been found to be only partially susceptible to a TAPI homologue (27).

In general, plasma CRP levels correlate with severity of inflammatory diseases. During the onset of inflammation or tissue injury, plasma concentrations of CRP are dramatically elevated from ~1 μg/ml in healthy individuals to as much as 500 μg/ml during the acute phase response (28).

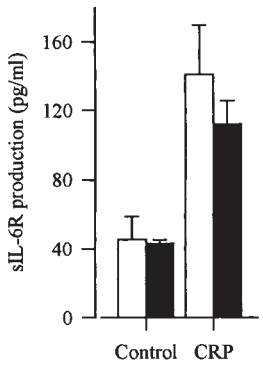

Figure 4. CRP-mediated sIL-6R production is partially inhibited by TAPI. Neutrophils (2×10^6 cells) were pretreated for 10 min with either 100 μM TAPI (black bars) or vehicle alone (white bars) at 37°C, 5% CO_2. Cells were stimulated for a further 40 min with 50 μg/ml CRP. Values are representative of three experiments and show mean sIL-6R production ± SD ($n = 3$).

In vitro studies have shown that control of this response is primarily regulated by IL-6 (29). More recently, human CRP-transgenic mice were used to verify in vivo that IL-6 is absolutely required for the induced expression of CRP during an inflammatory acute phase response (30). Our current findings show that this relationship between IL-6 and CRP is more complex than previously thought, since IL-6R shedding in response to CRP likely contributes to formation of the agonistic sIL-6R/IL-6 complex. Thus, CRP acts not only as an acute phase reactant, but it may have a profound effect on distal IL-6–mediated events that occur during the inflammatory process. Indeed, CRP levels in several diseases have been found to correlate with those of sIL-6R (31–33).

It is now recognized that CRP plays a significant role in host defense against pathogens (34). C-reactive protein also binds to specific receptors on human neutrophils and diminishes neutrophil responses, such as chemotaxis (35) and the activation of superoxide generation and degranulation by chemoattractants (36). In addition, CRP prevents neutrophil adhesion to endothelial cells via induction of L-selectin (CD62L) shedding (37). Consistent with these findings, in vivo studies have shown that CRP abates neutrophil recruitment in models of inflammation (38, 39). Taken together, these data indicate that CRP also performs an antiinflammatory function. It is therefore noteworthy that the sIL-6R/IL-6 complex has been shown to regulate proinflammatory activation of endothelial cells and to promote neutrophil recruitment (7, 8). In agreement with these findings, it has been observed that the extent of neutrophil infiltration into arthritic joints correlates with elevated sIL-6R levels in synovial fluid (40). It is conceivable that CRP may perform a pivotal role during inflammation by modulating the rate of neutrophil recruitment. It is also highly likely that CRP represents only one endogenous activator of IL-6R shedding, whereas release of DS–sIL-6R may also contribute to the overall properties of sIL-6R (13, 14, 18).

Previous studies have shown that peptides spanning residues 77–82 and 201–206 of the native CRP molecule block neutrophil superoxide generation and chemotaxis (23, 24), whereas peptide fragment 177–182 enhances cytokine/chemokine production and the tumoricidal activity of monocytic cells (41). Structure/function investigations of native CRP (for a review, see reference 34) reveal that amino acids 77–82 reside within the phosphocholine (PCh)-binding site of the CRP molecule, whereas residues 174–185 and 201–206 form parts of the walls of a deep cleft on the opposite face of the CRP protomer. The shallow end of this cleft represents the C1q-binding site of CRP (34), whereas residues 175–179 are important for Fcγ-R1 binding (42). Interestingly, in the present study, CRP peptides 174–185 and 201–206 effectively augmented sIL-6R production by human neutrophils. However, release was not observed in response to residues 77–82. Similarly, CRP peptides 174–185 and 201–206, but not 77–82, were found to mediate L-selectin shedding (37). These data argue that the ability of CRP to stimulate IL-6R and L-selectin shedding from neutrophils involves

interaction via the C1q/Fcg-R1 binding motif of CRP, and does not involve the PCh-binding site. Support for this concept is derived from the fact that disruption of the Ca^{2+}-dependent interaction of PCh with CRP (34) through the addition of EDTA had no effect on the CRP-induced release of sIL-6R (data not shown).

Although neutrophils express relatively high levels of the cognate IL-6R, IL-6 signaling in these cells is poorly defined and appears to evoke only weak biological activities (43, 44). However, shedding of the IL-6R from human neutrophils has been shown to activate endothelial cells (8). As a result, expression of the IL-6R on neutrophils may primarily serve as an inducible source of sIL-6R. Thus, the activated shedding of the IL-6R from neutrophils may indirectly propagate the inflammatory response via stimulation of resident tissue cells by the sIL-6R/IL-6 complex.

The authors wish to thank Drs. B.A. Freeman, J.S. Hagood, and V.B. O'Donnell for their help and advice. We are also grateful to Dr. R.A. Black for kindly providing TAPI, and Mrs. T. Rogers for FACScan® analysis.

This work was funded in part by an American Heart Association Research Fellowship awarded to S.A. Jones.

Address correspondence to Gerald M. Fuller, Department of Cell Biology, University of Alabama at Birmingham, Basic Health Science Bldg., 1918 University Blvd., Birmingham, AL 35294. Phone: 205-934-7596; Fax: 205-934-0950; E-mail: gmfuller@bmg.bhs.uab.edu

S.A. Jones's present address is School of Biosciences, University of Wales Cardiff, P.O. Box 911, Cardiff, CF1 3US, UK.

Received for publication 19 October 1998 and in revised form 4 December 1998.

References

1. Heinrich, P.C., I. Behrmann, G. Muller-Newen, F. Schaper, and L. Graeve. 1998. Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway. *Biochem. J.* 334: 297–314.
2. Rose-John, S., and P.C. Heinrich. 1994. Soluble receptors for cytokines and growth factors: their generation and biological function. *Biochem. J.* 300:281–290.
3. Hirota, H., K. Yoshida, T. Kishimoto, and T. Taga. 1995. Continuous activation of gp130, a signal-transducing receptor component for interleukin-6-related cytokines, causes myocardial hypertrophy in mice. *Proc. Natl. Acad. Sci. USA*. 92:4862–4866.
4. Mihara, M., Y. Moriya, T. Kishimoto, and Y. Ohsugi. 1995. Interleukin-6 (IL-6) induces the proliferation of synovial fibroblastic cells in the presence of soluble IL-6 receptor. *Br. J. Rheumatol.* 34:321–325.
5. Murakami-Mori, K.T., T. Taga, T. Kishimoto, and S. Nakamura. 1996. The soluble form of the IL-6 receptor (sIL-6Rα) is a potent growth factor for AIDS-associated Kaposi's sarcoma (KS) cells; the soluble form of gp130 is antagonistic for sIL-6R-induced AIDS-KS cell growth. *Int. Immunol.* 8:595–600.
6. Udagawa, N., N. Takahashi, T. Katagiri, T. Tamura, S. Wada, D.M. Findlay, T.J. Martin, H. Hirota, T. Taga, T. Kishimoto, and T. Suda. 1995. Interleukin (IL)-6 induction of osteoclast differentiation depends on IL-6 receptors expressed on osteoblastic cells, but not on osteoclast progenitors. *J. Exp. Med.* 182:1461–1468.
7. Romano, M., M. Sironi, C. Toniati, N. Polentarutti, P. Fruscella, P. Ghezzi, R. Faggioni, W. Luini, V. van Hinsbergh, S. Sozzani, et al. 1997. Role of IL-6 and its soluble receptor in induction of chemokines and leukocyte recruitment. *Immunity*. 6:315–325.
8. Modur, V., Y. Li, G.A. Zimmerman, S.M. Prescott, and T.M. McIntyre. 1997. Retrograde inflammatory signaling from neutrophils to endothelial cells by soluble interleukin-6 receptor alpha. *J. Clin. Invest.* 100:2752–2756.
9. Tilg, H., E. Trehu, M.B. Atkins, C.A. Dinarello, and J.W. Mier. 1994. Interleukin-6 (IL-6) as an anti-inflammatory cytokine: induction of circulating IL-1 receptor antagonist and soluble tumor necrosis factor receptor p55. *Blood.* 83:113–118.
10. Xing, Z., J. Gauldie, G. Cox, H. Baumann, M. Jordana, X.-F. Lei, and M.K. Achong. 1998. IL-6 is an antiinflammatory cytokine required for controlling local or systemic acute inflammatory responses. *J. Clin. Invest*. 101:311–320.
11. Müllberg, J., H. Schooltink, T. Stoyan, M. Gunther, L. Graeve, G. Buse, A. Mackiewicz, P.C. Heinrich, and S. Rose-John. 1993. The soluble interleukin-6 receptor is generated by shedding. *Eur. J. Immunol.* 23:473–480.
12. Müllberg, J., F.H. Durie, C. Otten-Evans, M.R. Alderson, S. Rose-John, D. Cosman, R.A. Black, and K.M. Mohler. 1995. A metalloprotease inhibitor blocks shedding of the IL-6 receptor and the p60 TNF receptor. *J. Immunol.* 155:5198–5205.
13. Lust, J.A.K., K.A. Donovan, M.P. Kline, P.R. Griepp, R.A. Kyle, and N.J. Maihle. 1992. Isolation of an mRNA encoding a soluble form of the human interleukin-6 receptor. *Cytokine*. 4:96–100.
14. Horiuchi, S., Y. Koyanagi, Y. Zhou, H. Miyamoto, Y. Tanaka, M. Waki, A. Matsumoto, M. Yamamoto, and N. Yamamoto. 1994. Soluble interleukin-6 receptors released from T cells or granulocyte/macrophage cell lines and human peripheral blood mononuclear cells are generated through an alternative splicing mechanism. *Eur. J. Immunol.* 24:1945–1948.
15. Kotake, S., K. Sato, K.J. Kim, N. Takahashi, N. Udagawa, I. Nakamura, A. Yamaguchi, T. Kishimoto, T. Suda, and S. Kashiwazaki. 1996. Interleukin-6 and soluble interleukin-6

receptors in the synovial fluids from arthritis patients are responsible for osteoclast-like cell formation. *J. Bone Miner. Res.* 11:88–95.
16. Kyrtsonis, M.C., G. Dedoussis, C. Zervas, V. Perifanis, C. Baxevanis, M. Stamatelou, and A. Maniatis. 1996. Soluble interleukin-6 receptor (sIL-6R), a new prognostic factor in multiple myeloma. *Br. J. Haematol.* 93:398–400.
17. Honda, M., S. Yamamoto, M. Cheng, K. Yasukawa, H. Suzuki, T. Saito, Y. Osugi, T. Tokunaga, and T. Kishimoto. 1992. Human soluble IL-6 receptor: its detection and enhanced release by HIV infection. *J. Immunol.* 148:2175–2180.
18. Walev, I., P. Vollmer, M. Palmer, S. Bhakdi, and S. Rose-John. 1996. Pore-forming toxins trigger shedding of receptors for interleukin-6 and lipopolysaccharide. *Proc. Natl. Acad. Sci. USA.* 93:7882–7887.
19. Cichy, J., S. Rose-John, J. Potempa, J. Pryjma, and J. Travis. 1997. Oncostatin-M stimulates the expression and release of the IL-6 receptor in human hepatoma HepG2 cells. *J. Immunol.* 159:5648–5653.
20. Jones, S.A., S. Horiuchi, D. Novick, N. Yamamoto, and G.M. Fuller. 1998. Shedding of the soluble IL-6 receptor is triggered by Ca^{2+} mobilization, while basal release is predominantly the product of differential mRNA splicing in THP-1 cells. *Eur. J. Immunol.* 28:3514–3522.
21. Novick, D., H. Engelmann, M. Revel, O. Leitner, and M. Rubenstein. 1991. Monoclonal antibodies to the soluble human IL-6 receptor: affinity purification, ELISA, and inhibition of ligand binding. *Hybridoma.* 10:137–146.
22. Shephard, E.G., S.M. Beer, R. Anderson, A.F. Strachan, A.E. Nel, and F.C. de Beer. 1989. Generation of biologically active C-reactive protein peptides by a neutral protease on the membrane of phorbol myristate acetate-stimulated neutrophils. *J. Immunol.* 143:2974–2981.
23. Shephard, E.G., R. Anderson, O. Rosen, M.S. Myer, M. Friedkin, A.F. Strachan, and F.C. de Beer. 1990. Peptides generated from C-reactive protein by a neutrophil membrane bound protease. *J. Immunol.* 145:1469–1476.
24. Heuertz, R.M., N. Ahmed, and R.O. Webster. 1996. Peptides derived from C-reactive protein inhibit neutrophil alveolitis. *J. Immunol.* 156:3412–3417.
25. Arribas, J., L. Coodly, P. Vollmer, T.K. Kishimoto, S. Rose-John, and J. Massagué. 1996. Diverse cell surface protein ectodomains are shed by a system sensitive to metalloprotease inhibitors. *J. Biol. Chem.* 271:11376–11382.
26. Mohler, K.M., P.R. Sleath, J.N. Fitzner, D.P. Cerretti, M. Alderson, S.S. Kerwar, D.S. Torrance, C. Otten-Evans, T. Greenstreet, K. Weerawarna, et al. 1994. Protection against a lethal dose of endotoxin by an inhibitor of tumour necrosis factor processing. *Nature.* 370:218–220.
27. Bennett, T.A., E.B. Lynam, L.A. Sklar, and S. Rogelj. 1996. Hydroxamic-based metalloprotease inhibitor blocks shedding of L-selectin adhesion molecule from leukocytes. Functional consequences for neutrophil aggregation. *J. Immunol.* 156: 3093–3097.
28. Pepys, M.B., and M.L. Baltz. 1983. Acute phase proteins with special reference to C-reactive protein and related proteins (pentaxins) and serum amyloid A protein. *Adv. Immunol.* 34:141–212.
29. Ganapathi, M.K., L.T. May, D. Schultz, A. Brabenec, J. Weinstein, P.B. Sehgal, and I. Kushner. 1988. Role of interleukin-6 in regulating synthesis of C-reactive protein and serum amyloid A in human hepatoma cell lines. *Biochem. Biophys. Res. Commun.* 157:271–277.
30. Szalai, A.J., F.W. van Ginkel, S.A. Dalrymple, R. Murray, J.R. McGhee, and J.E. Volanakis. 1998. Testosterone and IL-6 requirements for human C-reactive protein gene expression in transgenic mice. *J. Immunol.* 160:5294–5299.
31. Yokoyama, A., N. Kohno, Y. Hirasawa, K. Kondo, M. Abe, Y. Inoue, S. Fujioka, S. Fujino, S. Ishida, and K. Hiwada. 1995. Elevation of soluble IL-6 receptor concentration in serum and epithelial lining fluid from patients with interstitial lung diseases. *Clin. Exp. Immunol.* 100:325–329.
32. Kyriakou, D., H. Papadaki, A.G. Eliopoulos, A. Foudoulakis, M. Alexandrakis, and G.D. Eliopoulos. 1997. Serum soluble IL-6 receptor concentrations correlate with stages of multiple myeloma defined by serum beta 2-microglobulin and C-reactive protein. *Int. J. Hematol.* 66:367–371.
33. Mitsuyama, K., A. Toyonaga, E. Sasaki, O. Ishida, H. Ikeda, O. Tsuruta, K. Harada, H. Tateishi, T. Nishiyama, and K. Tanikawa. 1995. Soluble interleukin-6 receptors in inflammatory bowel disease: relation to circulating IL-6. *Gut.* 36:45–49.
34. Szalai, A.J., A. Agrawal, T.J. Greenhough, and J.E. Volanakis. 1997. C-reactive protein: structural biology, gene expression and host defense function. *Immunol. Res.* 16:127–136.
35. Kew, R.R., T.M. Hyer, and R.O. Webster. 1990. Human C-reactive protein inhibits neutrophil chemotaxis in vitro: possible implications for adult respiratory distress syndrome. *J. Lab. Clin. Med.* 115:339–345.
36. Filep, J., and E. Foldes-Filep. 1989. Effects of C-reactive protein on human neutrophil granulocytes challenged with *N*-formyl-methionyl-leucyl-phenylalanine and platelet-activating factor. *Life Sci.* 44:517–524.
37. Zouki, C., M. Beauchamp, C. Baron, and J. Filep. 1997. Prevention of in vitro neutrophil adhesion to endothelial cells through shedding of L-selectin by C-reactive protein and peptides derived from C-reactive protein. *J. Clin. Invest.* 100:522–529.
38. Heuertz, R.M., C.A. Piquette, and R.O. Webster. 1993. Rabbits with elevated serum C-reactive protein exhibit diminished neutrophil infiltration and vascular permeability in C5a-induced alveolitis. *Am. J. Pathol.* 142:319–328.
39. Ahmed, N., R. Thorley, D. Xia, D. Samols, and R.O. Webster. 1996. Transgenic mice expressing rabbit C-reactive protein exhibit diminished chemotactic factor-induced alveolitis. *Am. J. Respir. Crit. Care Med.* 153:1141–1147.
40. Desgeorges, A., C. Gabay, P. Silacci, D. Novick, P. Roux-Lombard, G. Grau, J.M. Dayer, T. Vischer, and P.A. Guerne. 1997. Concentrations and origins of soluble interleukin-6 receptor-α in serum and synovial fluid. *J. Rheumatol.* 24:1510–1516.
41. Barna, B.P., M.J. Thomassen, P. Zhou, J. Pettay, S. Singh-Burgess, and S.D. Deodhar. 1996. Activation of alveolar macrophage TNF and MCP-1 expression in vivo by a synthetic peptide of C-reactive protein. *J. Leukocyte Biol.* 59: 397–402.
42. Marnell, L.L., C. Mold, M.A. Volzer, R.W. Burlingame, and T.W. Du Clos. 1995. C-reactive protein binds to Fcγ-R1 in transfected COS cells. *J. Immunol.* 155:2185–2193.
43. Biffl, W.L., E.E. Moorte, F.A. Moore, C.C. Barnett, C.C. Silliman, and V.M. Peterson. 1996. Interleukin-6 stimulates neutrophil production of platelet-activating factor. *J. Leukocyte Biol.* 59:569–574.
44. Mullen, P.G., A.C. Windsor, C.J. Walsh, A.A. Fowler III, and H.J. Sugerman. 1995. Tumor necrosis factor-alpha and interleukin-6 selectively regulate neutrophil function in vitro. *J. Surg. Res.* 58:124–130.