

Glycosylation of acute phase proteins and interleukins following hip arthroplasty

Inflammation parameters studied in 10 patients

Klaus Fassbender^{1,2}, Bruno Gerber³, Urs Karrer², Magdalena Sobieska^{1,4},
Andre Aeschlimann² and Wolfgang Müller^{1,2}

We analyzed changes in glycosylation and serum concentrations of alpha₁-acid glycoprotein (AGP), antichymotrypsin (AC), interleukin-6 (IL-6), soluble interleukin-2 receptor (sIL-2R) and C-reactive protein (CRP) following hip arthroplasty. Glycosylation of AGP and AC showed an increased reactivity to concanavalin A between postoperative Day 2 and Day 5 and Day 10, respectively. Serum levels of AGP and

AC increased at the earliest on Day 5. The AC levels returned to baseline by Day 10. AGP, however, exhibited increased values beyond Day 14. CRP levels were elevated at Day 2 and remained increased beyond Day 14. sIL2R showed increased values at Days 5, 10 and 14. IL-6 was the first parameter to increase, and it returned to baseline in less than 5 days.

¹Hochrheinstitut of Rheumatism Research, Bad Säckingen, Germany; ²Departments of Rheumatology and ³Orthopedics, University Hospital, Basel, Switzerland; ⁴Department of Immunology and Rheumatology, University School of Medicine, Poznan, Poland

Correspondence: Dr. K. Fassbender, Department of Neurology, Mannheim, University of Heidelberg, Theodor-Kutzer-Ufer, D-6800 Mannheim 1, Germany. Tel +49-621 383 2885. Fax -621 383 3807

Submitted 92-05-15. Accepted 92-10-08

Changes in serum levels of acute phase proteins occur after major surgery (Benson and Hughes 1975, Shih et al. 1987, Kallio et al. 1990). Alpha₁-acid glycoprotein (AGP) and antichymotrypsin (AC) exhibit microheterogeneity, which is revealed by a different reactivity of subpopulations to concanavalin A. This reactivity depends on the content of biantennary oligosaccharides (Bog-Hansen 1973). An increase in the concanavalin A-reactive fraction is observed in acute infections (Hachulla et al. 1988, Nicollet et al. 1981, Fassbender et al. 1991, 1993) and after noninfected major surgery (Hansen et al. 1986). Glycosylation of acute phase proteins does not depend on serum levels; it represents a new acute phase phenomenon (Mackiewicz et al. 1989). Similarly, interleukin-6 (IL-6), a cytokine mainly produced by macrophages and monocytes (Bauer et al. 1988), rises in serum following infections (Helfgott et al. 1989), burns (Njisten et al. 1987) and surgical injury (Nishimoto et al. 1989, Shenkin et al. 1989). Soluble interleukin-2 receptors (sIL2R) respond also to acute burn injury (Xiao et al. 1988, Theodorczyk-Injeyan et al. 1989). This receptor, which is considered to be an early marker of immunostimulation (Robb et al. 1981), is expressed at the surface of activated T-cells (Robb and Greene 1983) and is released in its soluble form (Rubin et al. 1985). We studied the postoperative course of these recently described

parameters of inflammation after total hip replacement.

Patients and methods

Our series comprised 10 consecutive patients (7 women, 3 men) with a median age of 71 (53-88) years who had total hip arthroplasty for arthrosis at the Department of Orthopedics, University Hospital, Basel, Switzerland. Clinical examinations, chest roentgenograms, electrocardiography, erythrocyte sedimentation rate, serum levels of CRP, total blood cell count and urine analysis were performed to exclude infection. Blood samples were collected the day before operation, 2 and 6 hours after the first incision, and at 2, 5, 10, and 14 days, postoperatively.

Crossed affinity-immunoelectrophoresis and quantification of acute phase proteins, IL-6 and sIL-2R (Figure 1)

Agarose-based affinity-immunoelectrophoresis was carried out according to the description by Bog-Hansen (1973). Briefly, concanavalin A (Sigma type 4, lot 79 C-7620 Sigma, St. Louis, MO, U.S.A.) at 40

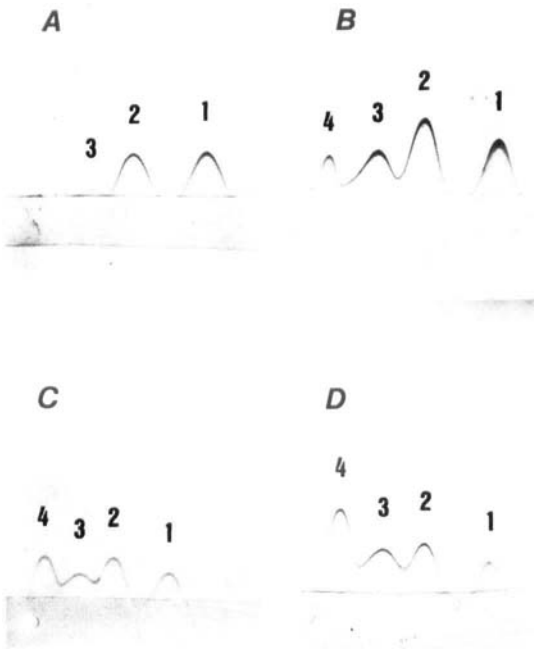


Figure 1. Crossed affinity-immunoelectrophoresis of α_1 -acid glycoprotein (A, B) and antichymotrypsin (C, D) using concanavalin A as ligand in sera from patients 1 day before (left) and 2 days following surgery (right). Postoperatively, the highly concanavalin A-reactive fractions (peaks 3 + 4) increased in relation to the less reactive fractions (peaks 1 + 2).

$\mu\text{mol/L}$, was incorporated into the first dimension gel. First-dimension electrophoresis lasted 1 hour (10 V/cm). The second-dimension gel contained 0.8% anti-AGP or anti-AC antibody (Atlantic Antibodies, Scarborough, ME, U.S.A.) and 7.5% alpha-methyl D-mannoside (Sigma). Migration was carried out for 20 hours (1.5 V/cm). This gel was then washed, dried, and stained with Coomassie blue R 250. The relative concentrations of the AGP- and AC-variants were determined by planimetry. Results were expressed as a AGP-concanavalin A-reactivity coefficient (AGP-RC) and AC-concanavalin A reactivity coefficient (AC-RC), which was calculated according to the formula: total area below the precipitation peaks of all concanavalin A-reactive subpopulations divided by the area of the most anodic peak corresponding to the nonreactive subpopulation (Mackiewicz et al. 1989). CRP was measured by an enzyme immunoassay (EMIT, C-reactive protein assay, Merck Diagnostica, Zurich, Switzerland). AGP- and AC-serum concentrations were assessed by rocket immunoelectrophoresis, as described by Laurell (1966). Serum IL-6 was meas-

ured with a commercially available quantitative "sandwich" enzyme immunoassay (R&D Systems Inc., MN, U.S.A.). This test involves a monoclonal antibody specific for IL-6 coated onto a microtiter plate and an enzyme-linked polyclonal antibody specific for IL-6, which is added after washing. The lower limit of detection is 5 pg/mL. sIL-2R was measured by means of a commercially available enzyme-linked immunosorbent assay (T-Cell Sciences, Cambridge, MA, U.S.A.) according to the method described by Rubin (1985). This sandwich-ELISA included the use of a monoclonal antibody against sIL-2R adsorbed onto the microtiter wells, and a second enzyme conjugated monoclonal antibody directed against a second epitope, binding to the IL-2R captured by the first antibody. Its detection limit is 50 U/mL.

Statistics

The data have been compared by the Friedmann 2-way ANOVA, and thereafter the Wilcoxon test was used.

Results (Figure 2)

The immediate postoperative course of all patients was without any complications. Glycosylation of AGP (preoperative AGP-RC: 1.18 ± 0.03) and AC (preoperative AC-RC: 3.92 ± 0.37) responded to surgical injury by showing an elevation of reactivity to concanavalin A on Day 2. The highly concanavalin A reactive fractions of AGP and AC increased after the operation. AGP-RC values normalized within 10 days, while increased AC-RC values persisted until Day 14. Serum levels of AGP (preoperative value: 1.13 ± 0.09 g/L) and AC (preoperative value: 0.52 ± 0.11 g/L) were increased at Day 5. Whereas serum concentrations of AC returned to normal by Day 10, elevated serum levels of AGP persisted beyond the observation period. Postoperative CRP concentrations (preoperative value: 3.7 ± 1.2 mg/L) were increased from Day 2 until Day 14. Levels of sIL-2R (baseline value: 496 ± 56 U/mL) were increased at Days 5, 10 and 14, although with a high variation of values (Figure 2). The preoperative value of IL-6 was 11.4 ± 3.0 pg/mL. Serum levels of this cytokine increased within 6 hours following the first incision and returned to baseline values in less than 5 days. Therefore, IL-6 was the first parameter to respond to surgery and the first to reach preoperative values.

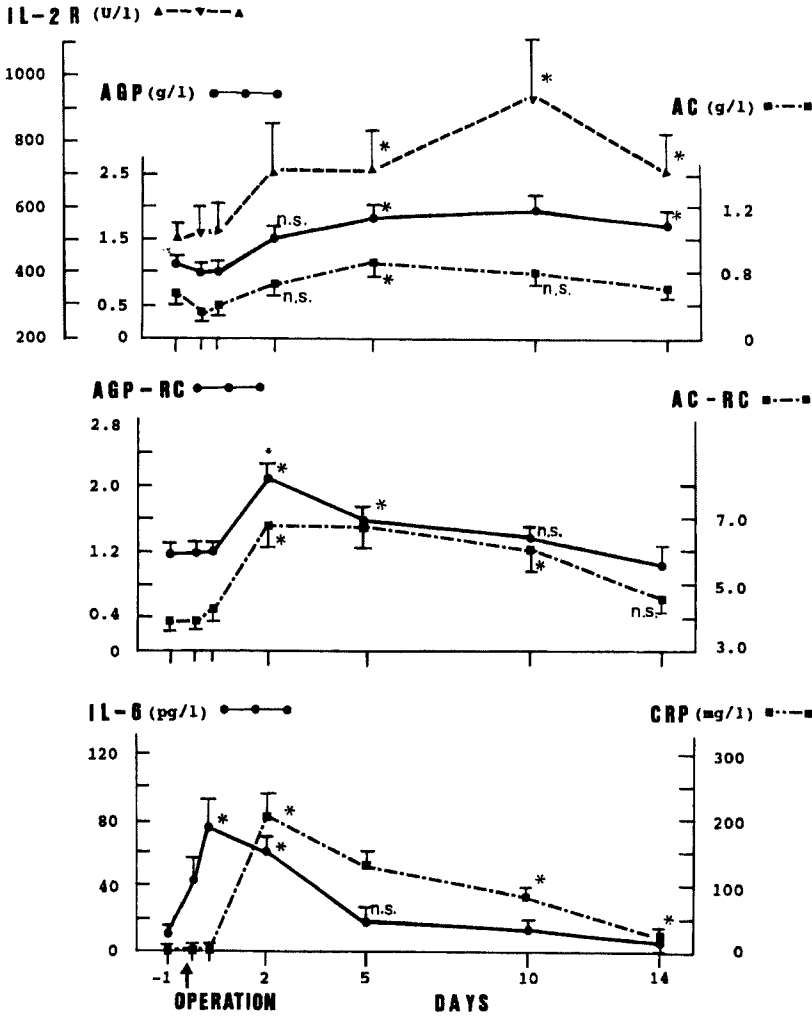


Figure 2. Mean values (+ SEM, vertical lines) of serum levels of soluble interleukin-2 receptor (sIL-2R), alpha1-acid glycoprotein (AGP), antichymotrypsin (AC), C-reactive protein (CRP), interleukin-6 (IL-6) and concanavalin A reactivity coefficients of alpha,-acid glycoprotein (AGP-RC) and antichymotrypsin (AC-RC) in 10 postoperative patients. AGP-RC and AC-RC are calculated according to the formula: Total area below the precipitation peaks of all concanavalin A-reactive subpopulations (peaks 2 + 3 + 4) divided by the area of the peak (peak 1) representing the non-reactive subpopulation (n.s. = not significant; * = $P < 0.05$).

Discussion

The results of our study comparing the postoperative kinetics of the glycosylation of acute phase proteins, serum IL-6, sIL-2R with the classical parameters of acute phase response demonstrate that only serum levels of IL-6 and the glycosylation of AGP were characterized by both, a short latency of response as well as a rapid return to baseline values.

The postoperative kinetics of the glycosylation changes and the serum levels of these acute phase proteins were different. Concanavalin A reactivity of

AGP and AC was increased 2 days following surgical injury, which indicated an increased proportion of biantennary oligosaccharides bound to these glycoproteins. AGP-RC, but not AC-RC, normalized already within 10 days. Serum concentrations of AC returned to normal by Day 10. In contrast, those of AGP persisted beyond the observation period.

As reported previously in patients undergoing hip arthroplasty (Shih et al. 1987) or tibial fractures (Kallio et al. 1990), the CRP response showed an elevation in serum levels until Day 14. However, normal values of CRP were found 1 week following lumbar dissec-

tomy (Kock-Jensen 1988), which may be explained by the smaller operative injury in this procedure.

IL-6 was the first parameter to decrease postoperatively, reaching baseline levels in less than 5 days. Although we used an immunologic method, our investigation confirmed recent reports of postoperative elevation in serum IL-6 levels before a rise in acute phase proteins (Nishimoto et al. 1989, Shenkin et al. 1989). These data are consistent with the previously-described role of IL-6 in mediating synthesis (Gauldie et al. 1987) and glycosylation (Mackiewicz et al. 1987) of acute phase proteins. SIL-2R increased slowly in response to surgery and did not normalize within the observation period. To our knowledge, this has not been described before and it would suggest that T-cell activation occurs during the acute phase response to surgical injury. IL-1, released by tissue damage, affects the hepatic synthesis of acute phase proteins (Dinarello 1984) as well as the release of interleukin-2 and its receptor (Kaye et al. 1984), which may offer an explanation for these data.

The postoperative response patterns of the parameters studied may be altered in patients with preexisting inflammatory diseases, such as infections or chronic rheumatic diseases, because of already increased preoperative values. However, patients with underlying inflammatory processes have been excluded in this study.

In conclusion, compared to the classical and new parameters of inflammation, glycosylation of AGP and IL-6 levels in serum are characterized by the most rapid postoperative increase and normalization. It seems possible that investigation of IL-6 and glycosylation of AGP may even be useful in the detection of inflammatory complications after surgery.

Acknowledgement

We would like to thank Mrs. Joyce Vogel for editorial assistance.

References

- Bauer J, Ganter U, Geiger T, Jacobshagen U, Hirano T, Matsuda T, Kishimoto T, Andus T, Acs G, Gerok W, et al. Regulation of interleukin-6 expression in cultured human blood monocytes and monocyte-derived macrophages. *Blood* 1988; 72 (4): 1134-40.
- Benson M K, Hughes S P. Infection following total hip replacement in a general hospital without special orthopaedic facilities. *Acta Orthop Scand* 1975; 46 (6): 968-78.
- Bog-Hansen T C. Crossed immunoaffinoelectrophoresis. An analytical method to predict the result of affinity chromatography. *Anal Biochem* 1973; 56 (2): 480-8.
- Dinarello C A. Interleukin-1 and the pathogenesis of the acute phase response. *N Engl J Med* 1984; 311 (22): 1413-8.
- Fassbender K, Zimmerli W, Kissling R, Sobieska M, Aeschlimann A, Kellner M, Muller W. Glycosylation of alpha-1 acid glycoprotein in relation to duration of disease in acute and chronic infections and inflammation. *Clin Chim Acta* 1991; 203 (2-3): 315-27.
- Fassbender K, Michels H, Zepp F, Zimmerli W, Aeschlimann A, Mackiewicz S, Truckenbrodt H, Müller W. Distinct patterns of glycosylation of alpha-1 acid glycoprotein in acute bacterial infection and systemic onset juvenile rheumatoid arthritis/Still's disease. *J Rheumatol* 1993. In press.
- Gauldie J, Richards C, Harnish D, Lansdorp P, Baumann H. Interferon beta-2/B cell stimulatory factor type-2 shares identity with monocyte-derived hepatocyte-stimulating factor and regulates the major acute phase protein response in liver cells. *Proc Natl Acad Sci U S A* 1987; 84 (20): 7251-5.
- Hachulla E, Laine A, Hayem A. Alpha-1 antichymotrypsin microheterogeneity in crossed immunoaffinoelectrophoresis with free concanavalin A: a useful diagnostic tool in inflammatory syndrome. *Clin Chem* 1988; 34 (5): 911-5.
- Hansen J E S, Jensen S P, Norgaard Pedersen B, Bog Hansen T C. Electrophoretic analysis of the glycan microheterogeneity of orosomucoid in cancer and inflammation. *Electrophoresis* 1986; 7: 180-3.
- Helfgott D C, Tatter S B, Santhanam U, Clarick R H, Bhardwaj N, May L T, Sehgal P B. Multiple forms of IFN beta-2/IL-6 in serum and body fluids during acute bacterial infection. *J Immunol* 1989; 142 (3): 948-53.
- Kallio P, Michelsson J E, Lalla M, Holm T. C reactive protein in tibial fractures. Natural response to the injury and operative treatment. *J Bone Joint Surg (Br)* 1990; 72 (4): 615-7.
- Kaye J, Gillis S, Mizel S B, Shevach E M, Malek T R, Dinarello C A, Lachman L B, Janeway C A Jr. Growth of a cloned helper T-cell line induced by a monoclonal antibody specific for the antigen receptor: interleukin-1 is required for the expression of receptors for interleukin-2. *J Immunol* 1984; 133 (3): 1339-45.
- Kock-Jensen C, Brandslund I, Sogaard I. Lumbar disc surgery and variations in C reactive protein, erythrocyte sedimentation rate and the complement split product C-3 d. *Acta Neurochir (Wien)* 1988; 90 (1-2): 42-4.
- Laurell C B. Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. *Anal Biochem* 1966; 15 (1): 45-52.
- Mackiewicz A, Ganapathi M K, Schultz D, Kushner I. Monokines regulate glycosylation of acute phase proteins. *J Exp Med* 1987; 166 (1): 253-8.
- Mackiewicz A, Khan M A, Reynolds T L, van der Linden S, Kushner I. Serum IgA, acute phase proteins, and glycosylation of alpha-1 acid glycoprotein in ankylosing spondylitis. *Ann Rheum Dis* 1989; 48 (2): 99-103.
- Nicollet I, Lebreton J P, Fontaine M, Hiron M. Evidence for alpha-1 acid glycoprotein populations of different pI values after concanavalin A affinity chromatography. Study of their evolution during inflammation in man. *Biochim Biophys Acta* 1981; 668 (2): 235-45.
- Nishimoto N, Yoshizaki K, Tagoh H, Monden M, Kishimoto S, Hirano T, Kishimoto T. Elevation of serum interleukin-6 prior to acute phase proteins on the inflammation by surgical operation. *Clin Immunol Immunopathol* 1989; 50 (3): 399-401.

- Njisten M W N, Degroot E R, Ten Duis H J, Klasen J, Hack C E, Aarden L A. Serum levels of interleukin-6 and acute phase responses. *Lancet* 1987; 2: 921.
- Robb R J, Munck A, Smith K A. T cell growth factor receptors. Quantitation, specificity, and biological relevance. *J Exp Med* 1981; 154 (5): 1455-74.
- Robb R J, Greene W C. Direct demonstration of the identity of T cell growth factor binding protein and the Tac antigen. *J Exp Med* 1983; 158 (4): 1332-7.
- Rubin L A, Kurman C C, Fritz M E, Biddison W E, Boutin B, Yarchoan R, Nelson D L. Soluble interleukin-2 receptors are released from activated human lymphoid cells in vitro. *J Immunol* 1985; 135 (5): 3172-7.
- Shenkin A, Fraser W D, Series J, Winstanley F P, McCartney A C, Burns H J, Van Damme J. The serum interleukin-6 response to elective surgery. *Lymphokine Res* 1989; 8 (2): 123-7.
- Shih L Y, Wu J J, Yang D J. Erythrocyte sedimentation rate and C reactive protein values in patients with total hip arthroplasty. *Clin Orthop* 1987; 225: 238-46.
- Teodorczyk-Injeyan J A, Sparkes B G, Mills G B, Falk R E, Peters W J. Increase of serum interleukin-2 receptor level in thermally injured patients. *Clin Immunol Immunopathol* 1989; 51 (2): 205-15.
- Xiao G X, Chopra R K, Adler W H, Munster A, Winchurch R A. Altered expression of lymphocyte II-2 receptors in burned patients. *J Trauma* 1988; 28 (12): 1669-72.