Intracellular Oxidative Activation in Synovial Fluid Neutrophils from Patients with Rheumatoid Arthritis But Not from Other Arthritis Patients

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ABSTRACT. ***Objective.*** To compare total and intracellular oxidative activation of blood and synovial fluid (SF) neutrophils from patients with rheumatoid arthritis (RA) and other arthritides with blood donor neutrophils.
Methods. Peripheral blood and SF samples were obtained from 26 gonarthritis patients (13 RA, 13 non-RA) attending the rheumatology unit for therapeutic joint aspiration. Isolated neutrophils were stimulated by a formylated tripeptide (fMLF) or by microbeads coated with collagen-I. Formation of superoxide-anion-derived reactive oxygen species (ROS) was studied by luminol-enhanced chemiluminescence. Paired samples of blood and SF neutrophils from patients with active arthritis were compared with blood neutrophils from patients in remission and from 47 healthy blood donors.
Results. SF neutrophils from patients with RA, but not from non-RA patients, showed high baseline intracellular ROS production. Blood neutrophils from arthritis patients in remission existed in a primed state as revealed by more rapid oxidative response after collagen-bead challenge and a more pronounced response after fMLF stimulation compared to healthy blood donors. Blood neutrophils from RA patients with ongoing gonarthritis, however, did not differ from healthy blood donors concerning oxidative activation, whereas blood neutrophils from non-RA patients with gonarthritis showed a significantly lower peak ROS production.
Conclusion. A novel finding with pathogenetic implications in our study is that SF neutrophils from patients with RA, but not other arthritides, are activated and produce ROS intracellularly. This implies that synovial neutrophils in RA are engaged in the processing of endocytosed material. (First Release Oct 15 2007; J Rheumatol 2007;34:2162–70)

Key Indexing Terms:
NEUTROPHILS ARTHRITIS REACTIVE OXYGEN SPECIES SUPEROXIDE ANION

In rheumatoid arthritis (RA) joints are invaded by leukocytes. Macrophages and lymphocytes dominate the inflammatory infiltrate in synovial tissue, whereas neutrophils predominate in synovial fluid (SF)[1]. For many years most research concerning the immunopathogenic mechanisms concentrated on the roles of T cells and macrophages in the synovial lining adjacent to cartilage and bone. In recent years, new interest has arisen regarding the importance of autoantibodies, immune complexes, complement, and their receptors[2-5]. Further, the interest in neutrophils and mast cells in arthritis is presently experiencing a renaissance. In K/BxN mice, for instance, autoantibodies, immune complexes, neutrophils, and mast cells play a crucial role for the development and persistence of erosive polyarthritis[6]. Also in SKG mice, which develop a spontaneous autoimmune polyarthritis resembling RA, neutrophils appear to play a prominent role in the disease process[7]. Granulocyte/monocyte apheresis has been reported to be an efficacious treatment option in RA[8].

The term "endocytosis" encompasses cellular uptake of soluble molecules/complexes (fluid-phase pinocytosis or receptor-mediated endocytosis) as well as particulate material (phagocytosis). Neutrophils are "professional phagocytes," meaning that they are specialized in binding, ingestion, and killing/degradation of particles, e.g., microbes. However, via Fc-gamma receptor (FcγR) ligation, neutrophils can also bind both soluble and insoluble IgG-containing immune complexes, which may result in oxidative activation and eventually endocytosis[9-13]. Attachment of neutrophils to cartilage, either directly or via tissue-trapped immune complexes, can induce degranulation and release of proteolytic enzymes[14]. NADPH-dependent activation of neutrophils, e.g., after binding/internalization of immune complexes, induces production of reactive oxygen species (ROS) such as superoxide anion ($\bullet O_2^-$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($\bullet OH^-$)[15]. Intracellular ROS production can thus be a sign of ongoing

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endocytosis and breakdown of ingested material, whereas extracellular release of ROS may contribute to tissue destruction, e.g., breakdown of articular connective tissue and release of molecules such as cross-linked carboxyterminal telopeptide of type I collagen and cartilage oligomeric matrix protein[16,17]. Despite their tissue-destructive potentials, it has been shown in genetically mutated rats and mice that deficient production of NADPH-dependent ROS increases the severity of T-cell-dependent RA-like arthritis[18,19], perhaps as a result of deficient ROS-dependent T-cell apoptosis during early maturation of the immune system[20]. Nitric oxide (NO•), which also belongs to the ROS family, is produced from L-arginine under the action of nitric oxide synthases, for instance by macrophages and neutrophils[21,22]. Together with $\bullet O_2^-$ it forms the very toxic ROS peroxynitrite[23]. Like NADPH-dependent ROS, NO• has tissue-destructive potentials as well as T-cell-modulating functions of importance in autoimmunity[24-26].

We investigated collagen-induced oxidative burst in blood neutrophils from healthy blood donors and from arthritis patients in clinical remission, as well as in blood and SF neutrophils from patients with RA and other arthritides, where therapeutic knee joint arthrocentesis was performed. The patients with arthritis were recruited consecutively with a "real-life approach" at our rheumatology outpatient clinic.

MATERIALS AND METHODS

Patients and controls. Twenty-six consecutive patients with acute gonarthritis attending the outpatient rheumatology clinic were judged to benefit from therapeutic joint aspiration. The patients were asked to participate in the study and all agreed. Thus, SF and blood samples were collected according to the established clinical routine. These samples were used for isolation of granulocytes (see below). Routine laboratory analyses (e.g., acute-phase reactants) were only performed if considered necessary by the physician. The 26 patients were divided into a group of 13 fulfilling ≥ 4/7 of the 1987 revised RA classification criteria according to the American College of Rheumatology[27] and 13 who did not. All patients were tested regarding the presence of latex-particle-agglutinating rheumatoid factor (RF) in serum.

The characteristics of the 26 patients with gonarthritis are summarized in Table 1.

Twenty-five arthritis patients in clinical remission (20 women, 5 men; mean age 55 yrs, range 24–79 yrs) and 47 healthy blood donors served as controls. Remission was defined as no signs of ongoing synovitis at clinical examination together with erythrocyte sedimentation rate (ESR) and plasma C-reactive protein (CRP) levels within normal limits. Sixteen of the patients in remission had RA, of which 13 were RF-positive. In the non-RA group, 3 patients had pelvospondylitis, 3 psoriatic arthritis, 2 undifferentiated spondyloarthropathy, and 1 juvenile idiopathic arthritis (oligoarthritic). Comparisons of the medication in the remission group versus the arthritic group did not reveal any major differences. Five patients were receiving treatment targeting tumor necrosis factor (anti-TNF), and 11 had methotrexate (mean dosage 14 ± 4 mg/wk). Four patients were treated with sulfasalazine, 2 hydroxychloroquine, 1 leflunomide, and 1 cyclosporine. Four patients were treated with ≥ 2 disease modifying antirheumatic drugs in combination. Six patients were treated with prednisolone (mean dose 5 ± 2 mg/day).

Table 1. Characteristics of the patients with active gonarthritis.

Sex	Age, yrs	Diagnosis*	Rheumatoid Factor	Medication (DMARD or glucocorticosteroids)**
M	63	RA	+	MTX 25 mg/wk, infliximab 300 mg/8 wks, prednisolone 7.5 mg/day
F	77	RA	+	Leflunomide 20 mg/day, prednisolone 5 mg/day
M	59	RA	+	MTX 15 mg/wk, infliximab 400 mg/wk, prednisolone 10 mg/day
F	72	RA	+	Etanercept 25 mg twice weekly, prednisolone 5 mg/day
M	47	RA	+	Etanercept 25 mg twice weekly, prednisolone 5 mg/day
M	31	RA	+	SSZ 2000 mg/day
F	65	RA	+	Prednisolone 5 mg/day
F	42	RA	+	Leflunomide 20 mg/day, prednisolone 7.5 mg/day
M	48	RA	+	Etanercept 25 mg twice weekly
F	64	RA	+	MTX 10 mg/wk, adalimumab 40 mg/2 wks, prednisolone 10 mg/day
F	51	RA	+	MTX 20 mg/wk
M	53	RA	–	Prednisolone 10 mg/day
F	36	RA	–	MTX 10 mg/wk, prednisolone 5 mg/day
M	49	ReA	–	MTX 15 mg/wk, prednisolone 5 mg/day
F	40	PsA	–	MTX 20 mg/wk
F	55	PsA	–	MTX 20 mg/wk, SSZ 2000 mg/day
M	55	AS	–	None
M	61	CPPD	–	None
F	24	UMA	–	None
M	67	UPA	–	MTX 15 mg/wk, SSZ 2000 mg/day
F	31	JIA, oligo	–	Azathioprine 200 mg/day, prednisolone 5 mg/day
M	30	JIA, oligo	–	MTX 20 mg/wk, etanercept 25 mg twice weekly
F	21	JIA, poly	–	None
F	34	JIA, oligo	–	Etanercept 25 mg twice weekly, prednisolone 5 mg/day
F	46	JIA, oligo	–	None
M	29	JIA, oligo	–	MTX 20 mg/wk, etanercept 25 mg twice weekly, prednisolone 5 mg/day

* AS: ankylosing spondylitis; CPPD: calcium pyrophosphate dihydrate deposition arthritis; JIA: juvenile idiopathic arthritis; oligo: oligoarticular; poly: polyarticular; PsA: psoriatic arthritis; RA: rheumatoid arthritis; ReA: reactive arthritis; UMA: undifferentiated monoarthritis; UPA: undifferentiated polyarthritis. ** MTX: methotrexate; SSZ: sulfasalazine.

Isolation of neutrophils. Venous blood was drawn into heparinized test tubes. Density gradients were formed by layering 5 ml Lymphoprep® (Axis-Shield, Oslo, Norway) over 20 ml Polymorphprep® (Axis-Shield). Twenty-five ml blood was added on top and centrifuged at 480 × g for 40 min at room temperature. The neutrophil-rich layer was removed and washed with phosphate buffered saline (PBS, pH 7.3). Contaminating red blood cells were lysed in water for 30 s. The cells were resuspended in ice-cold Krebs-Ringer glucose buffer (KRG) without Ca^{2+} and centrifuged at 200 × g for 10 min at 4°C. The lysis step was repeated once and the cells were then resuspended to 1×10^7 cells/ml in KRG supplemented with Ca^{2+} (1 mmol l^{-1}).

SF was centrifuged at 600 × g for 10 min. Pellets were resuspended in PBS and layered on top of 25 ml Lymphoprep. After centrifugation at room temperature for 40 min at 480 × g, the neutrophils in the pellets were resuspended in KRG with Ca^{2+} and adjusted to a concentration of 1×10^7 cells/ml.

The purity and viability of isolated cells were checked using flow cytometry (FACS Calibur, Becton Dickinson, Franklin Lakes, NJ, USA) and 2 commercial kits for detection of apoptosis, Annexin V-FITC Apoptosis detection kit and Apo-Direct™ Kit (BD Biosciences, San Jose, CA, USA). The purity (judged by granularity) was always > 95% for blood cells. The average purity of SF cells was 90% (range 80–98%). The viability was always > 97% for both blood and SF cells. All samples were used for experiments within 60 min.

Oxidative activation. NADPH-dependent oxidative activation of granulocytes was studied by luminol-enhanced chemiluminescence in a 6-channel Biolumat LB 9505 (Berthold Co., Wildbad, Germany). 0.95 ml KRG-suspended granulocytes ($1 \times l0^6$ cells/ml) were stimulated at 37°C by incubation with 50 µl of a priming agent in KRG, either a suspension of nonphagocytoseable microcarrier beads coated with acid-extracted denatured collagen type I (Cytodex-3, Amersham Biosciences, Uppsala, Sweden), or as a control, 0.1 µM solution of a formylated tripeptide (fMLF, Sigma Chemical Co., St. Louis, MO, USA). The use of Cytodex-3 beads to study neutrophil activation has been described[28]. To enhance measurement of extracellularly released ROS in case of extracellular shortage of peroxidase, 4 U horseradish peroxidase (Roche, Palo Alto, CA, USA) was used. Measurement of intracellularly generated ROS was done by using 200 U superoxide dismutase (Roche), i.e., a cell-impermeable scavenger for superoxide anion, and 2000 U catalase (Roche), a cell-impermeable scavenger for hydrogen peroxide. All samples were light-emission enhanced with 20 µg/ml luminol (5-amino-2,3-dihydro-1,4-phatalazinedione; Sigma), a cell-permeable CL substrate. After equilibration in 37°C for 5 min, the stimulus was added. The light emission was recorded continuously, 15 min for fMLF samples and 30 min for Cytodex-3 stimulated samples.

Statistics. Comparisons of groups were done using peak values, and Student's t-test was used for the statistical analyses unless otherwise stated.

The study protocol was approved by the regional ethics committee in Linköping.

RESULTS

Arthritis patients in remission versus healthy blood donors. As shown in Figure 1A, blood neutrophils from arthritis patients in clinical remission (RA and non-RA grouped together) had a significantly more rapid oxidative response to Cytodex-3 stimulation compared to healthy blood donor neutrophils, regarding total (peroxidase-enhanced) as well as intracellular ROS production. At half peak-time for the control cells (7.2 min) the arthritis-derived neutrophils had reached > 90% of their peak activity. At this timepoint, the chemiluminescence response was $1.82 \pm 0.26 \times 10^8$ counts per minute (cpm) from the control cells and $2.67 \pm 0.28 \times 10^8$ cpm from the arthritis cells ($p < 0.05$). The total amount of ROS produced (curve integrals in Figure 1A) did not differ. fMLF stimulation generated an oxidative response with identical kinetics in arthritis patients and healthy blood donors (Figure 1B), but here the magnitude of ROS production was significantly higher ($p < 0.001$) by neutrophils from patients with arthritis, both totally (peroxidase-enhanced, 4.25 ± 0.4 vs $0.95 \pm 0.24 \times 10^8$ cpm) and intracellularly (6.6 ± 0.38 vs $1.2 \pm 0.1 \times 10^7$ cpm, not illustrated).

Patients with active gonarthritis. Grouped together, peripheral blood as well as SF neutrophils from arthritis patients with active gonarthritis (13 RA and 13 non-RA arthritis patients) showed significant differences compared to blood neutrophils from healthy donors. Thus, circulating neutrophils from patients with gonarthritis responded with a significantly lower average peak (3.2 ± 0.24 vs $4.0 \pm 0.3 \times 10^8$ cpm; $p < 0.01$) upon Cytodex-3 stimulation compared to healthy blood donor cells, although the kinetics were similar (not illustrated). The peak values generated in peripheral blood neutrophils after fMLF stimulation did not differ between patients with arthritis and blood donors, but a more prolonged response was evident in the arthritic patients [$p < 0.01$ comparing curve integrals (not illustrated)]. SF neutrophils, on the other hand, showed higher average baseline activity and responded with a significantly higher total output of ROS upon challenge with Cytodex-3 ($p < 0.01$) as well as with fMLF ($p < 0.001$) compared to healthy blood donor cells (not illustrated).

Subgrouping of the 26 gonarthritis patients revealed that the lower peak activity noted in blood neutrophils after Cytodex-3 stimulation could be pinpointed down to the non-RA cells (4.0 ± 0.3 vs $2.6 \pm 0.3 \times 10^8$ cpm; $p < 0.05$), whereas neutrophils from patients with RA did not differ from the controls (Figure 2A). A raised baseline activity was found in SF neutrophils from patients with RA (Figure 2B). Thus, compared to healthy blood donor neutrophils, SF cells from patients with RA had significantly higher baseline activity (0.07 ± 0.06 vs $1.7 \pm 0.9 \times 10^8$ cpm; $p < 0.009$), whereas the non-RA patients' neutrophils did not differ significantly from the control cells ($p = 0.44$). In contrast, although the non-RA SF neutrophils had low baseline activity they tended to react with a more rapid response upon stimulation with Cytodex-3 ($p < 0.09$ at 2 min after subtraction of baseline activities in each group; Figure 2B). The fMLF-stimulated controls confirmed elevated baseline activity and prolonged response upon stimulation in RA SF neutrophils but no differences were observed concerning the rapidity of the response in any cell populations (not illustrated).

Further analyses of total (peroxidase-enhanced) and intracellular Cytodex-3-induced response in RA SF neutrophils revealed that the elevated baseline activity was clearly intracellular since it also could be observed in the presence of superoxide dismutase and catalase (Figure 3A). Unlike peripheral blood neutrophils from healthy donors, reaching maximum intracellular ROS production after 20–30 min, stimulation with Cytodex-3 induced a further elevation of intracellular activity within a few minutes in SF neutrophils from RA patients (Figure 3B). This rapid Cytodex-3-induced

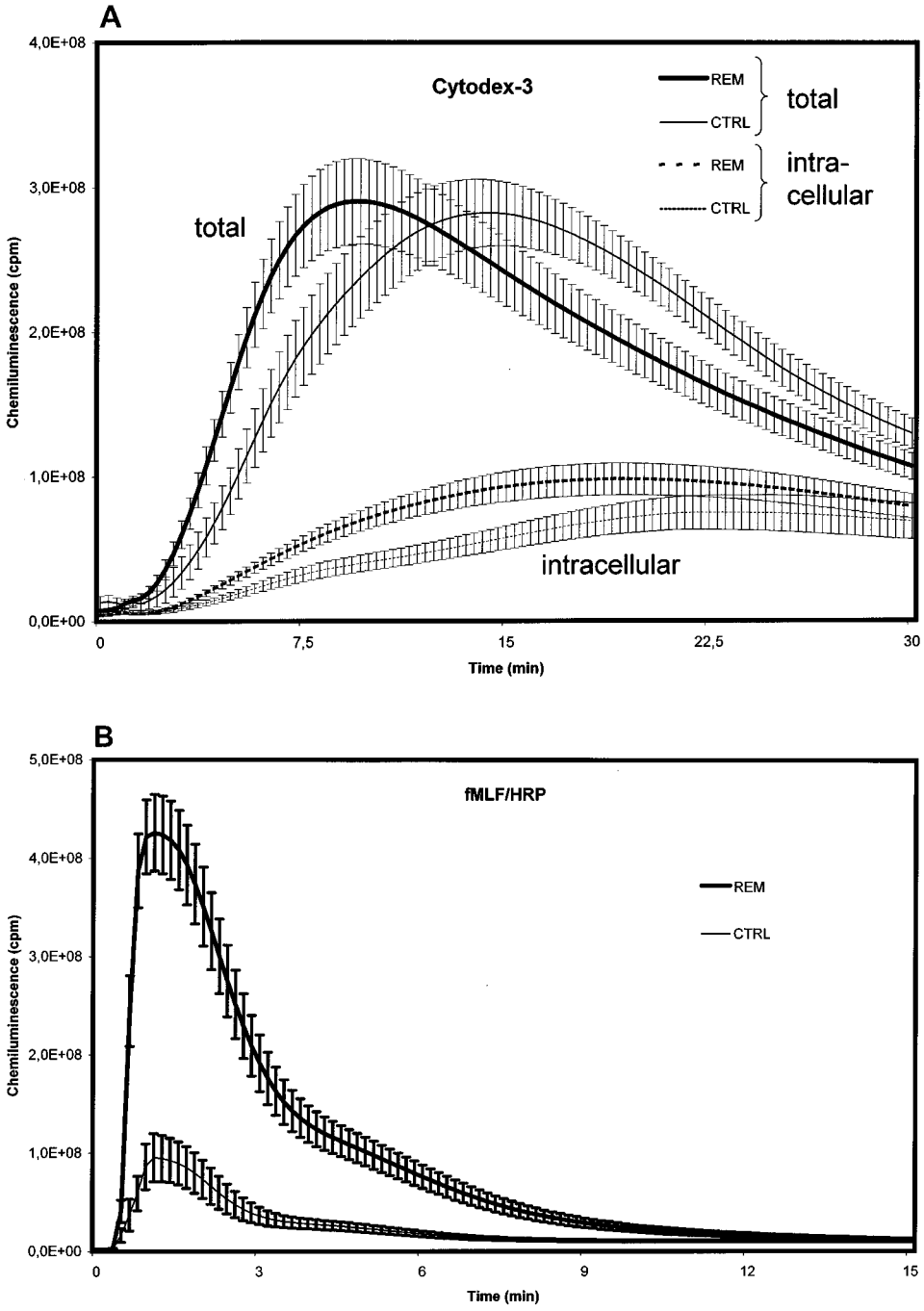

Figure 1. Oxidative activation of peripheral blood neutrophils from arthritis patients in remission (REM) and blood donors (CTRL). Mean chemiluminescence (CL) values ± SEM. A. Total (enhanced by horseradish peroxidase, HRP) and intracellular (SOD and catalase-treated) CL responses after Cytodex-3 stimulation. RA and non-RA grouped together (n = 20), and compared with healthy blood donors (n = 44). In both cases the response (peak value) was significantly more rapid in cells from patients with arthritis ($p < 0.05$). B. Total (HRP-enhanced) CL responses after fMLF activation of cells from 12 patients with arthritis and 12 healthy blood donors. The differences in peak as well as integral values were significant ($p < 0.001$ in both instances).

response was not observed after stimulation with fMLF (not illustrated) and contrasted to the limited intracellular chemiluminescence responses in all other neutrophil populations 3.3 min after stimulus, i.e., the timepoint for peak intracellular activity in RA SF neutrophils ($p < 0.05$ vs non-RA SF neutrophils after subtraction of baseline activity; Figure 3B). Circulating neutrophils from RA patients with ongoing synovitis also showed significant increase in intracellular activation compared to non-RA patients ($p = 0.02$ at peak activity; Figure 3B).

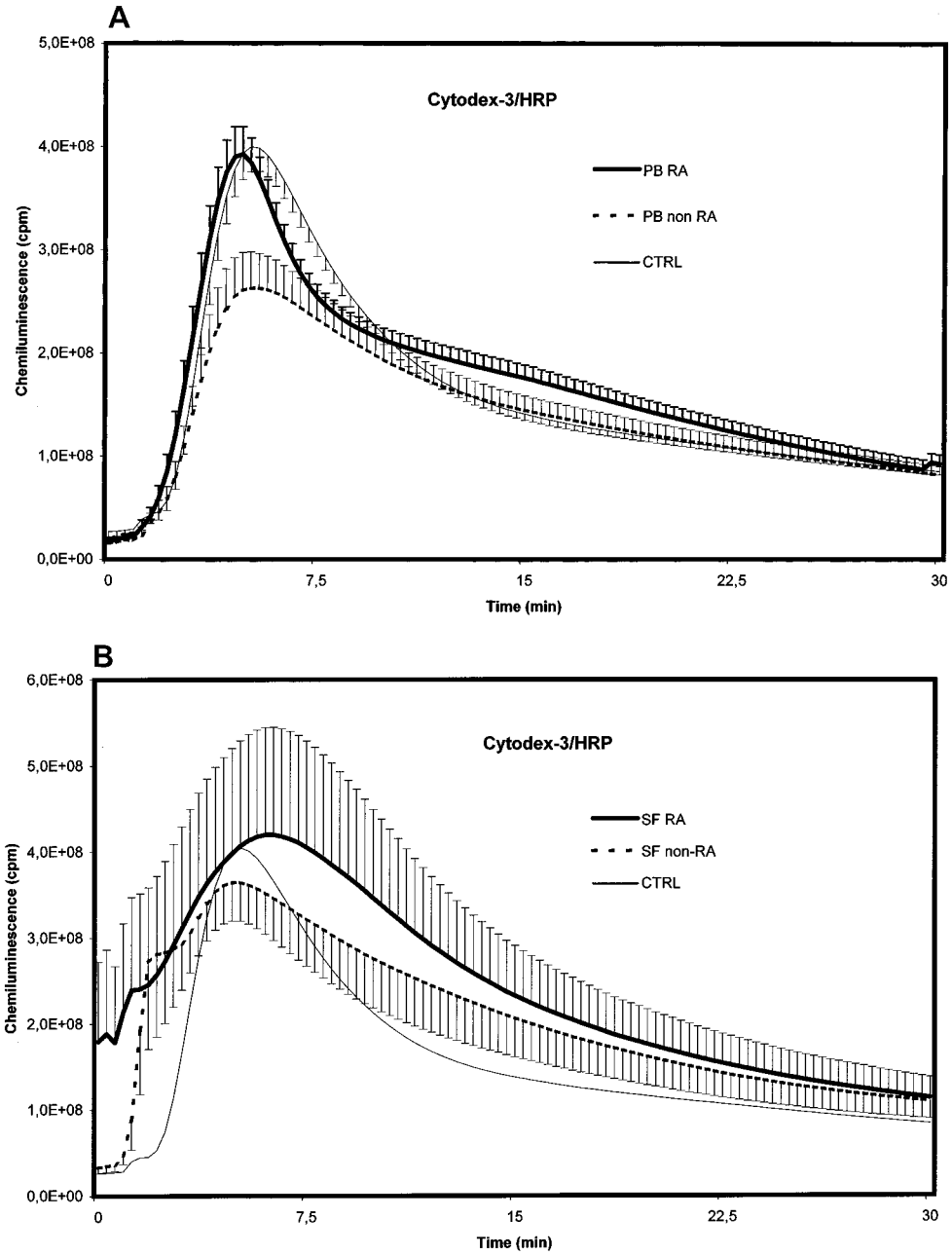

Figure 2. Cytodex-3-induced oxidative activation in paired neutrophil samples from synovial fluid (SF) and peripheral blood (PB) in RA and non-RA patients with ongoing synovitis and in healthy blood donors (CTRL). Data represent mean ± SEM. A. Total (HRP-enhanced) chemiluminescence response in peripheral blood neutrophil samples from 13 patients with RA, 13 non-RA patients, and 35 blood donors. The kinetics did not differ among the 3 populations. Neutrophils from patients with RA produced the same total amount of ROS as healthy controls, whereas the non-RA patients had a significantly lower peak value ($p < 0.01$). B. Total CL response in SF neutrophil samples. RA neutrophils have a significantly higher baseline activity ($p < 0.009$), whereas non-RA neutrophils respond more rapidly upon activation ($p < 0.09$).

Subgrouping for anti-TNF treatment did not reveal any differences in addition to those obtained by stratifying according to diagnosis. No effect of TNF-inhibitor treatment was noted concerning oxidative activation in circulating neutrophils. In SF neutrophils we noted lower baseline activity and diminished response in some anti-TNF-treated patients, but the numbers studied in each group were small and the differences did not reach statistical significance.

DISCUSSION

The importance of humoral immunity, phagocytes, and their receptors for IgG-Fc (FcγR) and complement in the pathogen-

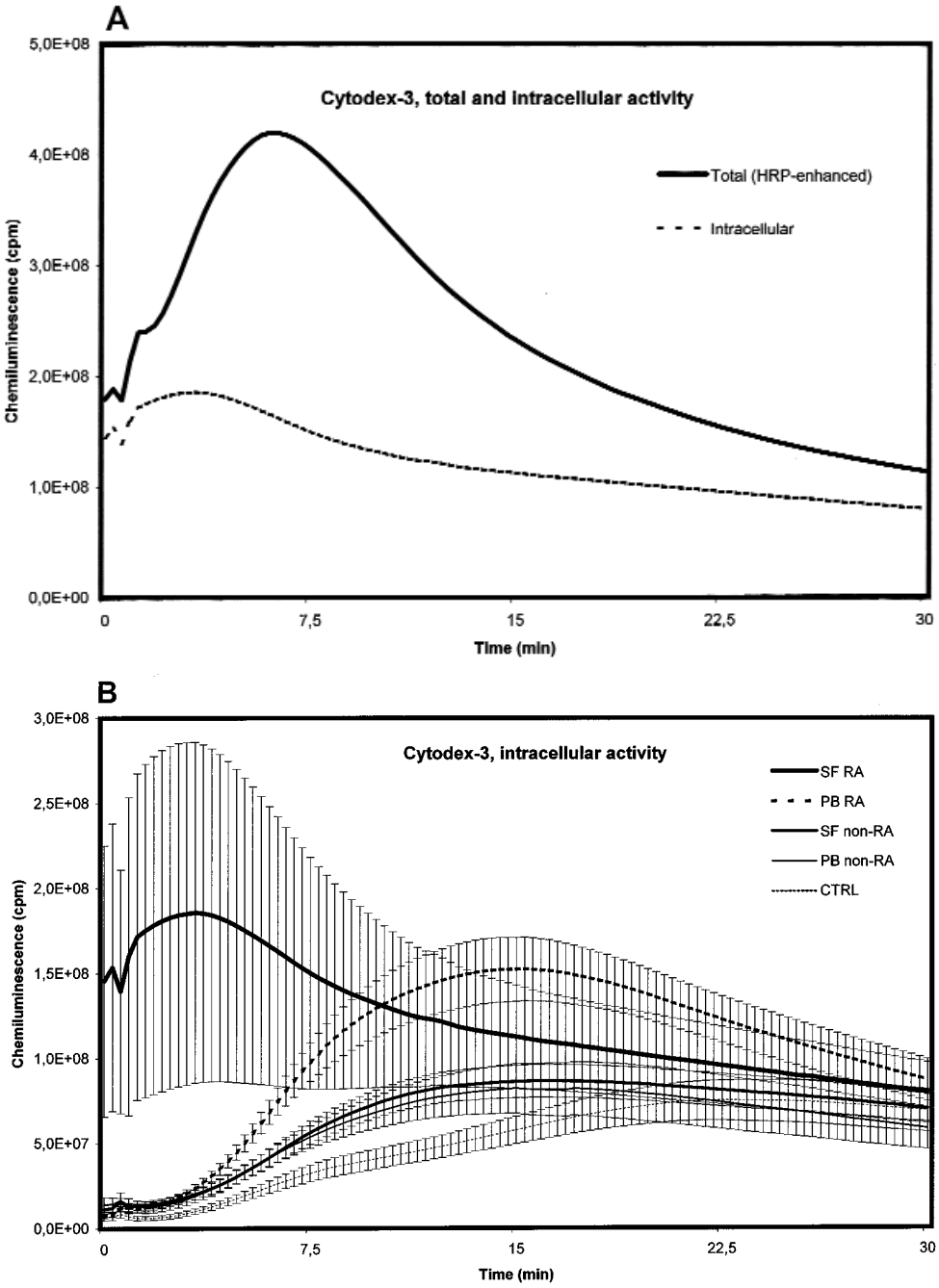

Figure 3. A. Total (HRP-enhanced) and intracellular (superoxide dismutase and catalase treatment) mean oxidative activity after Cytodex-3 stimulation of SF neutrophils from 13 patients with RA. The raised baseline activity is almost exclusively intracellular. B. Intracellular oxidative activity in the same SF neutrophils depicted in (A) and peripheral blood (PB) neutrophils from the same patients. The graph also shows SF and PB activity in 13 non-RA arthritis patients as well as in PB neutrophil samples from 35 healthy blood donors (CTRL). The graphs represent mean values ± SEM. RA SF neutrophils clearly exhibit both higher baseline activity and a more rapid response after stimulation.

esis of arthritis has been highlighted in many recent reports. In our study on neutrophil involvement in human arthritis, we present novel data indicating a distinct oxidative activation pattern in neutrophils from patients with RA.

The role of neutrophil oxidative activation in the pathogenesis of RA was the subject of many studies in the 1980s and 1990s[9-12,29,30]. Conflicting results concerning oxidative burst patterns are probably mainly due to methodological differences, including methods of cell isolation, choice of stimuli, and the techniques to assess oxygen metabolite production and release[31-34].

In a series of experiments, Robinson and coworkers chal-

lenged healthy blood donor neutrophils with soluble and insoluble immune complexes isolated from SF of patients with RA. They found that oxidative activation by soluble immune complexes required interaction with FcγRIII in cooperation with FcγRII, whereas oxidative activation by the insoluble immune complexes was essentially independent of FcγRIII and only partly reliant on FcγRII[9]. In several animal models, e.g., the spontaneously occurring autoimmune arthritis in K/BxN mice, neutrophils, immune complexes, FcγR, and complement receptors have been shown to be of crucial importance for the induction and chronicity of the disease[6].

Besides their cornerstone position in innate immunity, it has become evident that neutrophils orchestrate early adaptive immune responses in several ways[35-37]. In light of this, we find it interesting that SF neutrophils in our study showed different oxidative responses depending on whether they were derived from patients with RA or patients with other arthritides. The majority (85%) of the RA patients with gonarthritis in our study were RF-positive, all of which were also positive regarding antibodies to cyclic citrullinated peptide (CCP2, data not shown), whereas all non-RA gonarthritis patients were RF-negative. Although intriguing, the effects of RF on immune-complex handling still remain largely speculative. Due to RF-binding to exposed Fc parts in IgG-containing immune complexes, their removal by FcγR ligation can be impaired[38]. On the other hand, by the same mechanism, immune-complex mediated activation of phagocytes may also be ameliorated by RF[39]. Nevertheless, it has been shown that RF-mediated aggregation of small IgG immune complexes can enhance the oxidative activation of neutrophils[40]. A unique feature of RA-derived SF neutrophils, as demonstrated in our study, is the elevated intracellular ROS production at baseline. This is in keeping with the reports that SF from patients with RA, but not from other subjects, induces oxidative activation due to the presence of IgG-containing immune complexes[9,40,41]. With this in mind, our results suggest that joint fluid neutrophils in RA may be engaged in the processing of immune complexes. A likely explanation to immune-complex formation in RA joints, distinguishing it from other arthritides, is the strong link to antibodies to citrullinated proteins/peptides along with the presence of citrullinated antigens[42] and RF in the inflamed joints. Of course, other mechanisms may also be operative and further studies are required to shed more light on the intraarticular neutrophil activation in RA.

Collagen is the only protein known to be fragmented by superoxide anion[43] and this oxygen radical, as well as other NADPH-dependent ROS, may be important in the tissue destruction in RA[44]. Apart from its presence in eyes and nucleus pulposus of intervertebral disks, collagen type II is essentially restricted to hyaline cartilage, where it constitutes ~80% of the collagen content[45]. Collagen type II has therefore attracted great interest as a target antigen for specific immune responses in experimental autoimmune arthritis and in RA[46]. Collagen type I is widely distributed and localized corresponding to the organ involvement seen in RA, e.g., bone, tendons, skin, eyes, and organ capsules[45], and as a reflection of pathological degradation, synovial as well as serum levels of cross-linked carboxyterminal telopeptide of type I collagen are raised in patients with RA[11]. Collagen type I binds and activates neutrophils via the αLß2 integrin[47], and this accounts also for the microcarrier collagen-type-I-coated beads used in our study (unpublished results). Therefore, it is tempting to speculate that a self-perpetuating circle between collagen type I and neutrophil activation can emerge. By stimulating neutrophils with particle-bound denaturated collagen type I in our study, we found interesting differences between the intracellular ROS production in neutrophils from patients with RA compared to neutrophils from patients with other arthritides and blood donors.

The fact that circulating neutrophils in arthritis patients are primed[48,49], as also demonstrated in our study, may hypothetically be an effect of antirheumatic medication, e.g., by preventing homing of primed cells to the joint, but could possibly also reflect the constitution of the patients with arthritis. An additional finding in our study, confirming the results of others[32,50], was that SF neutrophils in patients with gonarthritis produced more ROS than blood neutrophils from the same patients on the same occasion. This too may indicate either active recruitment of primed circulating cells to the joint and/or local efficient priming of SF neutrophils. Although the number of patients treated with TNF inhibitors was too small to allow clear conclusions, our results accord with the findings in den Broeder, *et al*[51] and Capsoni, *et al*[52], who found that such therapy did not affect peripheral blood neutrophil ROS production. This, however, does not exclude the possibility that TNF inhibitors affect neutrophil functions in several ways, including oxidative activation[53].

Although the method of leukocyte isolation (Lymphoprep combined with a lysis step) may have affected the results, this is not likely to explain the distinct results observed in different subject strata. Comparing blood donor neutrophils and blood neutrophils from arthritis patients in clinical remission with paired samples of blood and SF neutrophils from patients with active arthritis, we found that circulating cells from the patients with active synovitis produced less ROS than blood neutrophils from healthy blood donors and arthritis patients in clinical remission. We also found that the SF neutrophils in active arthritis produced more fMLF- as well as Cytodex-3-mediated ROS compared to the circulating cells, probably reflecting active recruitment of primed cells from the blood to the joint. Comparison of RA patients with other arthritis patients revealed that cells from those with RA on average responded with more potently, indicating a more severe disease.

Our study shows that circulating neutrophils from arthritis patients in clinical remission are primed for stimulation via both integrins and the fMLF pathway, and that neutrophils in RA and non-RA arthritis differ with respect to oxidative activation patterns. In RA, the SF neutrophils have high intracel-

lular production of ROS at baseline, indicating processing of ingested material. Further studies on autoantibody-positive (RF and anti-CCP) and autoantibody-negative RA are warranted.

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