CD64 on Neutrophils Is a Sensitive and Specific Marker for Detection of Infection in Patients with Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** In inflammatory diseases, differentiation between infection and disease flares is often clinically difficult because of similar signs and symptoms, such as fever and elevation of inflammatory markers. In rheumatoid arthritis (RA), infection is not only one of the major complications but also one of the frequent causes of death. Use of biologic agents such as tumor necrosis factor-α blockers has been reported to increase the incidence of tuberculosis or opportunistic infections. We examined the utility of CD64 (FcγRI) expressed on neutrophils as a marker for detection of infection complicated with RA.

Methods. We measured the expression level of CD64 per neutrophil quantitatively by flow cytometry in 279 samples from 237 patients with RA with various levels of disease activity or types of infection, and in 52 samples from 36 controls including subjects with infection.

Results. CD64 expression was significantly higher among RA patients with infection (median 4156 molecules per neutrophil, interquartile range 2583–8587) than in those without infection (884, IQR 670–1262) ($p < 0.001$). The sensitivity of CD64 on neutrophils for the diagnosis of infection (using a cutoff value of 2000 molecules per cell) was 92.7% and specificity was 96.5%. CD64 expression was not affected by the disease activity of RA or the use of corticosteroids, disease modifying antirheumatic drugs, and biologic agents. CD64 was upregulated in infection by bacteria, viruses, fungi, and mycobacteria.

Conclusion. Our results suggest that quantitative measurement of CD64 expression on neutrophils can be used as a sensitive and specific marker to detect infection complicating RA. (First Release Oct 15 2006; J Rheumatol 2006;33:2416–24)

Key Indexing Terms:
RHEUMATOID ARTHRITIS INFECTION CD64 NEUTROPHIL

Infection is one of the major complications in patients with rheumatoid arthritis (RA). The hazard ratio for infection in RA compared with healthy controls has been reported to be 1.70[1]. In addition, infection is a major cause of death in RA[2,3]. This may be due to the immunomodulatory effects of RA itself or the use of immunosuppressive agents for RA treatment. A population-based study indicated that the risk factors for infection were increasing age, presence of extraarticular manifestations, leukopenia, corticosteroid use, and concomitant comorbidities (e.g., chronic lung disease, alcoholism, organic brain disease, and diabetes)[4]. Recently, concerns about infection in RA have become heightened because of reports of opportunistic infections or tuberculosis in patients treated with biologic agents[5-7]. So far, the rates of infection seen during clinical trials of tumor necrosis factor (TNF-α) blockers (etanercept, infliximab, and adalimumab) in RA have not significantly increased compared with those in the placebo groups[8-11]. However, recent reports suggest that the incidence of tuberculosis[12,13] and a variety of other infectious diseases is increased in patients with RA treated with TNF-α blockers[14].

Patients with RA complicated by infection may present initially with signs and symptoms of nonspecific inflammation such as fever, malaise, and polyarthralgia, which were also present in patients experiencing RA flare. Further, elevations

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of C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) cannot differentiate between infection and RA flare. Radiological findings or other diagnostic techniques such as imaging lack sensitivity in the early stage of infection. Microbiological cultures or polymerase chain reaction (PCR) testing of specimens are time-consuming and often lead to false-negative results. Because the treatment differs dramatically, differentiation between a disease flare and infection in a patient with RA is important and often urgent at bedside, but sometimes very difficult clinically. Therefore, a highly specific and sensitive infection marker that can provide results rapidly and easily is desirable for distinguishing infection from a flare of disease in RA.

CD64 (FcγRI), one of the Fc receptors for IgG, plays a role in antibody-dependent cytotoxicity, clearance of immune complexes, and phagocytosis of targets opsonized with IgG, and also mediates release of cytokines including interleukin 1 (IL-1), IL-6, and TNF-α. CD64 is constitutively expressed on macrophages and monocytes and is upregulated on neutrophils as a physiological response to microbial wall components such as lipopolysaccharide (LPS), complement split products, and some cytokines such as interferon-γ (IFN-γ)[15] and granulocyte colony stimulating factor (G-CSF)[16]. Upregulation of CD64 expression on neutrophils occurs within 4 to 6 hours after stimulation with IFN-γ, G-CSF, or LPS. In RA, it was reported that CD64 expression on the peripheral neutrophils was within normal limits in patients with active disease as well as inactive patients[17]; however, CD64 expression on neutrophils in the synovial fluid was increased in patients with active disease[18]. In infection, CD64 expression was reported to be induced not only by bacteria[19-22] but also by viruses[19] and mycobacteria[23].

Based on these observations, several reports had shown that upregulation of CD64 on neutrophils is a useful diagnostic marker of infection, especially in infants[20-22]. Recently, Allen, *et al* reported that neutrophil CD64 expression could distinguish adult patients with acute inflammatory autoimmune disease from those with systemic infections[24]. However, it remains unclear whether CD64 can be useful to distinguish infection from a disease flare in patients with autoimmune inflammatory diseases including RA.

We quantitatively measured the expression level of CD64 on neutrophils in patients with RA with various disease activities and in those whose disease was complicated by various types of infection, and evaluated whether neutrophil CD64, as a marker, could differentiate between infection and RA flare. Further, we measured neutrophil CD64 expression longitudinally in 3 patients with RA treated with biologic agents (2 patients with infliximab and one patient with etanercept) to confirm the clinical utility of CD64 for differentiating RA flare and infection.

MATERIALS AND METHODS

Patients. Patients for the first study were monitored at Sagamihara National Hospital from January to June, 2004. Samples were collected from consecutive inpatients and outpatients treated at the Department of Rheumatology. For the first study, 279 peripheral blood samples were collected from 237 patients with RA who met the 1987 revised criteria of the American College of Rheumatology[25]. They included 6 patients treated with biologic agents (infliximab 2, etanercept 1, abatacept 3). A previous study indicated that CD64 expression on neutrophils was upregulated by vasculitis[24]. Further, our preliminary study indicated that CD64 expression was upregulated by noninfectious interstitial pneumonia in RA (data not shown). Based on this background, RA patients were divided into 3 groups: Group 1 (simply called "RA"): patients with neither vasculitis nor noninfectious active interstitial pneumonia (219 patients; 185 women, 34 men; mean age 61.1 yrs, age range 18–84); Group 2 ("RA-V"): patients with vasculitis (12 patients; 11 women, 1 man; mean age 62.4 yrs, range 51–78); and Group 3 ("RA-IP"): patients with noninfectious active interstitial pneumonia (6 patients; 5 women, 1 man; mean age 68.0 yrs, range 60–74).

Interstitial pneumonia was diagnosed by high-resolution computed tomography. All the patients in Group 3 were improved by administration of high-dose corticosteroid without antimicrobial, antiviral, or antifungal agents. Of the 6 patients in Group 3, 3 had methotrexate (MTX)-related interstitial pneumonia and the other 3 had RA-related interstitial pneumonia. There were 217 samples from patients without infection (201 from RA, 10 from RA-V, and 6 from RA-IP) and 62 from patients whose disease was complicated by infection (56 from RA and 6 from RA-V). Infection was defined as follows: (1) pathogen was proven by microbiologic culture or PCR; (2) infection was diagnosed by radiological findings or other imaging diagnostics by more than 2 physicians; or (3) patient had obvious symptoms of infection (e.g., cough and purulent sputum with fever for respiratory infection, local heat, redness, and swelling with pus of skin for skin infection, pollakisuria and residual urine with pyuria for urinary tract infection) and/or obvious effects of treatment (improvement of symptoms shown above) with antimicrobial, antiviral, or antifungal agents were observed clinically. As controls, 52 samples from 36 healthy subjects were examined (30 women, 6 men; mean age 62.3 yrs, range 27–91). Controls were selected from healthy hospital personnel to match for age and sex with the patients. There were 40 samples from controls without infection and 12 samples with infection. Controls had no underlying disease including RA.

Blood samples used for this study were the unused portions of samples obtained for routine blood tests, and were not collected only for this study. CD64 was measured once per subject during the period. In some RA patients and controls, samples were collected both during infection and when there was no infection. All samples from patients and controls with infection were collected during active periods of infection and before starting treatments for the infection. For the second study, serial blood samples were collected from 3 RA patients treated with biologic agents (2 patients with infliximab, 1 with etanercept).

The study was approved by the medical ethics committee of Sagamihara National Hospital and all subjects gave consent.

Quantitative measurement of CD64 expression. CD64 expression on neutrophils was measured by staining whole blood with QuantiBrite CD64PE/CD45PerCP (Becton-Dickinson, San Jose, CA, USA) according to the manufacturer's instructions. Briefly, 20 μl of QuantiBrite CD64PE/CD45PerCP was added to 50 μl of whole blood and incubated for 60 min in the dark at room temperature. This was followed by lysis of red blood cells with 2 ml of 1× FACS™ lysing solution (Becton-Dickinson), without washing, followed with an additional 60 min incubation to reduce nonspecific background staining[21]. These specimens were analyzed using a FACScan flow cytometer (Becton-Dickinson) calibrated with QuantiBrite PE beads (Becton-Dickinson). The QuantiBrite PE beads contain 4 different beads with known numbers of phycoerythrin (PE) molecules that make it possible to create a standard curve for determining the mean number of PE molecules present on a cell. As the CD64-PE antibody has been designed to bind one PE molecule per antibody, the mean number of CD64 molecules expressed on cells can be calculated using the PE fluorescence quantification kit with QuantiBrite PE beads. The 3 different cell populations (lymphocytes, mono-

cytes, and granulocytes) were identified and gated by their CD45/side-scatter profile.

Statistical analysis. The SigmaStat statistical program (SPSS Science, Chicago, IL, USA) was used for statistical analysis. CD64 values on neutrophils were reported as medians and interquartile range (IQR). Comparisons of the expression levels of CD64 on neutrophils were made using the Mann-Whitney U test for evaluation of pairwise differences and the Kruskal-Wallis test for evaluation of differences between groups. The correlation coefficient was obtained using simple regression analysis. The optimal cutoff value for the CD64 expression level was determined from the receiver-operator curve (ROC) analysis.

RESULTS

Neutrophil CD64 expression in RA. Among subjects without infection, CD64 expression on neutrophils in RA (Group 1: median 884, IQR 670–1262) did not differ significantly from that of controls (median 864, IQR 753–1026); however, CD64 expression in RA-V (Group 2: median 1390, IQR 1058–2606) differed significantly from those in RA and in controls. Among subjects with infection, the median CD64 expression in the 3 groups, that is, controls, RA, and RA-V, was 4130 (IQR 1514–11136), 4156 (2583–8587), and 3219 (2427–7137), respectively. The expression was significantly increased compared with those without infection, but CD64 expression levels among subjects with infection in each group did not differ from other groups significantly. CD64 expression among patients with noninfectious active interstitial pneumonia (RA-IP) (median 5002, IQR 2646–8367) was significantly higher than in those without infection in both RA and RA-V, but did not differ significantly from those with infection.

Relationship of CD64 level to disease activity in RA. To determine whether the disease activity of RA affects the expression level of CD64 on neutrophils, we compared the CD64 expression with the level of CRP in RA patients (Group 1; Figure 2). In RA patients with infection, CD64 expression was correlated significantly with CRP ($r = 0.378$, $p = 0.004$) and tended to be upregulated even if CRP was not above the normal range. On the other hand, in RA patients without infection, CD64 expression did not increase even though CRP was elevated by the high activity of RA itself.

Cutoff level of CD64 for detection of infection complicated with RA. With ROC evaluation using the above results from RA (Group 1), a level of CD64 > 2000 molecules per neutrophil was found to be sensitive and specific for detection of infection in RA patients without vasculitis or noninfectious active interstitial pneumonia. This cutoff level of CD64 was the same as that which could differentiate systemic infection from autoimmune diseases reported in a previous study[24]. A CD64 level of 2000 was found to have a sensitivity of 92.7% (CD64 > 2000 in 51 of 55 patients with infection) and speci-

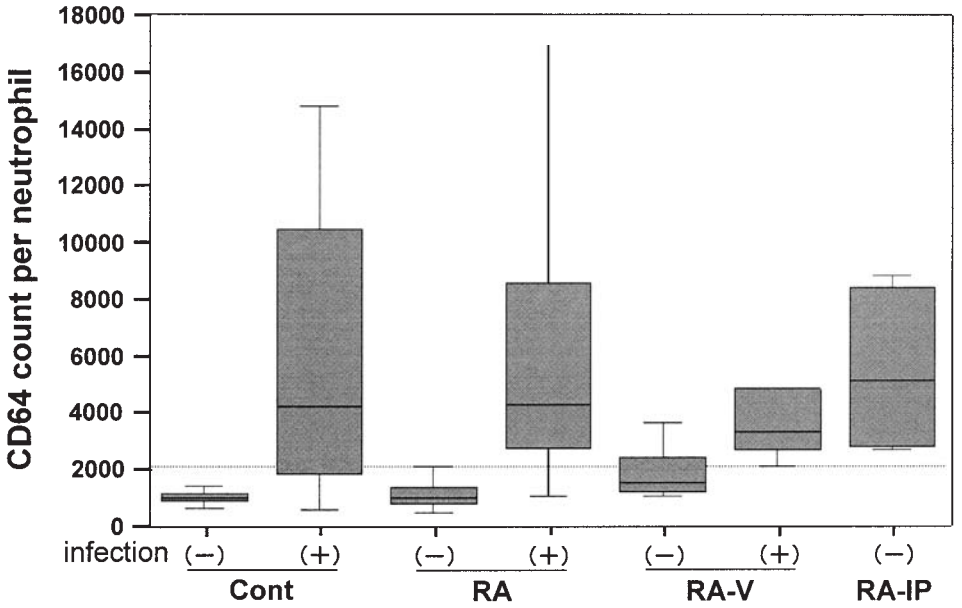

Figure 1. Expression of CD64 molecules per neutrophil in RA. Groups were defined as "RA": RA with neither vasculitis nor noninfectious active interstitial pneumonia; "RA-V": RA with vasculitis; and "RA-IP": RA with noninfectious active interstitial pneumonia. Line inside the box indicates the median value; box shows the 25th and 75th percentiles, and bars indicate 10th and 90th percentiles. Broken line shows the cutoff point for presence of infection in RA, i.e., > 2000 CD64 molecules per neutrophil. P values are as follows: control (–) vs control (+), $p < 0.001$; control (–) vs RA (–), not significant (NS); control (–) vs RA-V (–), $p < 0.001$; control (–) vs RA-IP, $p < 0.001$; control (+) vs RA (+), NS; control (+) vs RA-V (+), NS; control (+) vs RA-IP, NS; RA (–) vs RA (+), $p < 0.001$; RA (–) vs RA-V (–), $p < 0.005$; RA (–) vs RA-V (+), $p < 0.001$; RA (–) vs RA-IP, $p < 0.001$; RA (+) vs RA-V (–), $p < 0.001$; RA (+) vs RA-V (+), NS; RA (+) vs RA-IP, NS; RA-V (–) vs RA-V (+), $p < 0.05$; RA-V (–) vs RA-IP, $p < 0.005$; RA-V (+) vs RA-IP, NS.

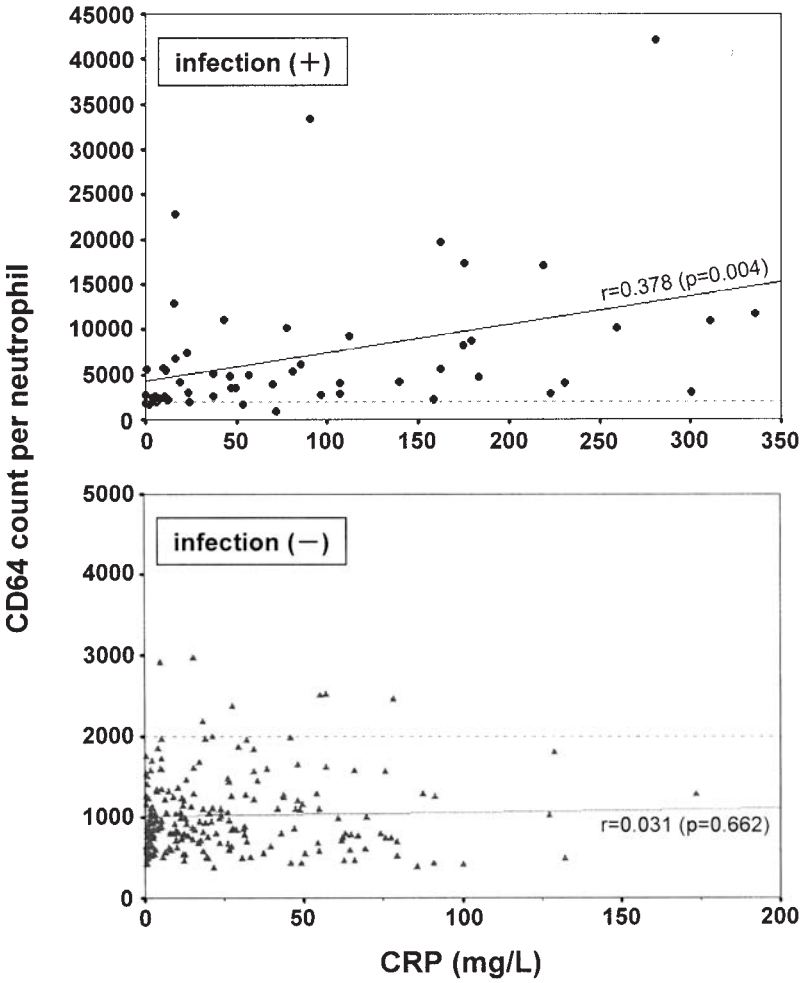

Figure 2. Comparison of CD64 expression on neutrophils with CRP in RA. Data represent RA patients with infection (upper panel) and without infection (lower panel). Broken line shows the cutoff point for infection in RA: 2000 CD64 molecules per neutrophil.

ficity of 96.5% (CD64 < 2000 in 195 of 202 patients without infection).

Pathogens and expression levels of CD64. A variety of infections were observed among subjects with infection whose CD64 expression was over 2000 molecules per neutrophil (Tables 1 and 2). The types of infection were diverse, and CD64 was upregulated by various kinds of pathogens including bacteria, mycobacteria, viruses, and fungi. Further, CD64 was upregulated in RA patients regardless of the use of corticosteroids, disease modifying antirheumatic drugs (DMARD) of any kind, or biologic agents. Comparing the expression levels of CD64 among subjects with infection, CD64 expression in tuberculosis tended to be higher compared to any other type of pathogen, although the difference was not statistically significant ($p = 0.063$ by Kruskal-Wallis test; Figure 3).

Clinical utility of measurements of CD64 expression in RA. To evaluate the clinical utility of neutrophil CD64 for detection of infection in RA, we measured CD64 expression longitudinally in patients with RA, especially in those treated with biologic agents. Data from a representative case are presented in Figure 4.

Patient S was a woman with RA. Her disease activity remained very high even after treatment with prednisolone plus MTX or other DMARD. The CRP level was elevated (> 100 mg/l), nonetheless, the CD64 level was low, less than 1000 per neutrophil. She started to receive infliximab and responded immediately. CRP level decreased dramatically and remained around 10 mg/l. Periodic treatments with infliximab kept disease activity, CRP, and CD64 expression level stable. Interruption of treatment with infliximab because of a cholecystectomy for gallstones worsened the RA disease activity, with elevation of CRP (up to 120 mg/l); however, CD64 expression remained less than 1000 per neutrophil. Resumption of treatment with infliximab improved her RA disease activity, and CRP level was again reduced. Treatment with infliximab kept her RA stable without adverse effects, but her left gonalgia was not improved, because of knee joint destruction. Therefore, she underwent a left total knee arthro-

Table 1. CD64 expression levels in pathogen-proven infections.

Sample No.	Disease	Age Sex	Pathogen	Group	Type of Infection	CD64	CRP, mg/l	Steroid, mg	DMARD/Biologic Agent
RA1	RA	78 F	M. tuberculosis	Mycobacterium	Miliary TB	33407	90.5	PSL 10	—
Cont1	Control	81 F	M. tuberculosis	Mycobacterium	Miliary TB	28000	15.6	—	—
RA2	RA	77 M	M. tuberculosis	Mycobacterium	Pulmonary TB	19745	162.2	PSL 10	—
RA3	RA	66 F	M. avium	Mycobacterium	Pneumonia	6758	16.4	PSL 7	Leflunomide 20 mg
RA4	RA	83 F	Strep. pneumoniae	Gram-pos	Bronchitis	1720	2.0	PSL 5	MTX 6mg/wk, bucillamine 50 mg
Cont2	Control	27 F	Staph. epidermidis	Gram-pos	Phlegmone	14689	167.8	—	—
RA5	RA	78 F	Staph. epidermidis	Gram-pos	Spondylitis	2990	300.3	PSL 4	Cyclosporine 100 mg
RA6	RA	84 F	MRSA	Gram-pos	Sepsis	17301	175.4	PSL 5	
RA7	RA	84 F	MRSA	Gram-pos	Pneumonia	10893	310.9	PSL 5	—
RA8	RA	70 M	MSSA	Gram-pos	Sepsis	4069	230.6	PSL 10	—
RA9	RA	66 F	L. monocytogenes	Gram-pos	Meningitis	7486	22.8	PSL 5	—
RA10	RA	83 F	E. coli	Gram-neg	Sepsis	11799	335.9	PSL 7	—
RA11	RA	81 F	E. coli	Gram-neg	Cholecystitis	10198	77.3	mPSL 3	—
RA12	RA	62 F	E. coli	Gram-neg	Cholecystitis/sepsis	4852	46.2	PSL 10	MTX 4 mg/wk
RA13	RA	74 F	E. coli	Gram-neg	Sepsis	4712	183.1	—	—
RA14	RA	68 F	P. aeruginosa	Gram-neg	UTI	3942	70.0	PSL 22.5	—
RA15	RA	60 F	P. aeruginosa	Gram-neg	Bronchitis	3537	47.0	mPSL 3	MTX 4 mg/wk
RA16	RA	56 F	Salmonella paratyphi B	Gram-neg	Colitis	17097	219.3	PSL 8	MTX 6 mg/wk
RA 17	RA	78 F	Varicella-zoster virus	Virus	Herpes zoster	22849	16.1	PSL 30	MTX 8 mg/wk
RA18	RA	29 F	Varicella-zoster virus	Virus	Herpes zoster	4157	19.0	—	MTX 6 mg/wk
RA19	RA	72 F	Varicella-zoster virus	Virus	Herpes zoster	2261	11.7	PSL 7	Leflunomide 20 mg
Cont3	Control	36 M	Influenzavirus	Virus	Influenza	11897	NT	—	—
RA20	RA	54 F	Influenzavirus	Virus	Influenza	5553	0.5	PSL 4	—
RA21	RA	74 F	Influenzavirus	Virus	Influenza	2914	222.6	mPSL 2	Bucillamine 200 mg, SASP 500 mg
RA 22	RA	62 F	Cytomegalovirus	Virus	Pneumonia	8192	174.5	PSL 10	—
RA 23	RA	74 F	Cytomegalovirus	Virus	Pneumonia	5514	11.0	PSL 5	SASP 1000 mg
RA 24	RA	70 F	P. carinii	Fungus	Pneumonia	5360	80.9	PSL 30	—
RA 25	RA	62 F	A. fumigatus	Fungus	Pneumonia	4033	106.6	—	—
Cont4	Control	67 F	A. fumigatus	Fungus	Pneumonia	3750	63.5	—	—
RA-V1	RA with vasculitis	66 F	C. albicans	Fungus	Esophagitis	3000	25.6	PSL 17.5	SASP 1000 mg

UTI: urinary tract infection, PSL: prednisolone, mPSL: methylprednisolone, MTX: methotrexate, SASP: salazosulfapyridine, NT: not tested, MRSA: methicillin-resistant S. aureus.

plasty. CRP was transiently elevated after the surgery, but CD64 expression was not affected. Afterwards, she had an upper respiratory tract infection of *Streptococcus pneumoniae*, with increased CD64 expression on neutrophils as well as upregulation of CRP. Administration of antibiotics improved her infection, with decreases of CRP and CD64. This treatment series and associated measurements noted above indicated the clinical utility of CD64 to detect infection regardless of the influences of biologic agents, RA disease activity, and total knee arthroplasty.

DISCUSSION

In inflammatory diseases including RA, infection is often hard to detect because fever and the elevation of inflammatory markers such as CRP or ESR are nonspecific signs and symptoms and do not enable physicians to distinguish infection from a flare of the underlying disease. The utility of CD64 to detect infection has been reported in subjects without underlying diseases[20-22] and in those with inflammatory diseases including a small number of patients with RA[24]; however, this is the first report demonstrating that CD64 is a useful marker for infection in patients with RA, regardless of its disease activity.

Quantitative measurements of CD64 can be performed easily and rapidly (within 2 hours) in any laboratory with flow cytometry facilities. We have confirmed that the test can be performed in less than 1 hour by our modified protocol for staining (data not shown). In addition, the cost for this test is not high (about $30 US per test in our laboratory) compared with other inflammatory markers. Considering these advantages, this test can be easily and broadly used for the routine clinical detection of infection. However, the number of facilities using flow cytometry is limited. Our preliminary study indicated that expression of CD64 on neutrophils is fairly stable for at least 24 hours (data not shown). Therefore, measurement of CD64 could be contracted to an outside laboratory or company.

Upregulation of CD64 on neutrophils was observed in

Table 2. CD64 expression levels in pathogen-unproven infections.

Sample No.	Disease	Age Sex	Type of Infection	CD64	CRP, mg/l	Steroid, mg	DMARD/Biologic Agent
RA26	RA	57 F	Organizing pneumonia	42082	280.8	—	Infliximab 3 mg/kg, MTX 8 mg/wk
RA27*	RA	46 F	Pneumonia	10207	259.2	PSL 2.5	MTX 4 mg
Cont5	Control	91 M	Pneumonia	3091	47.1	—	—
RA28	RA	72 F	Pneumonia	2553	37.3	mSPL 6	—
RA29	RA	79 F	Bronchitis	5124	37.2	mPSL 2	—
RA30	RA	64 M	Bronchitis	4971	56.8	PSL 10	SASP 1000 mg, auranofin 6 mg
RA31	RA	54 F	Bronchitis	2772	0.3	PSL 4	—
RA32	RA	67 F	Bronchitis	2427	6.1	—	MTX 8 mg/wk
RA33	RA	68 F	Bronchitis	2175	158.5	PSL 8	—
RA34	RA	54 F	Bronchitis	1795	0.2	PSL 3	—
RA35	RA	26 F	URI	12872	15.9	—	SASP 250 mg
RA36	RA	73 F	URI	9231	112.0	PSL 10	—
RA37	RA	54 F	URI	5753	10.1	PSL 5	MTX 4 mg, SASP 1000 mg
Cont6	Control	27 F	URI	4583	NT	—	—
Cont7	Control	38 M	URI	4509	2.9	—	—
RA38	RA	73 F	URI	2973	23.5	PSL 8	—
RA39	RA	54 F	URI	2848	NT	PSL 4	—
RA40	RA	61 F	URI	2553	5.5	mPSL 4	Infliximab 3 mg/kg, MTX 4 mg/wk
RA41	RA	53 F	URI	2473	4.2	PSL 6	MTX 8 mg/wk
RA42	RA	63 M	URI	2331	8.1	mPSL 2	Leflunomide 20 mg
RA43	RA	63 F	URI	2269	12.5	—	GST 25 mg/m
Cont8	Control	48 M	URI	2062	4.4	—	—
RA44	RA	60 F	URI	2018	6.0	mPSL 2	MTX 6 mg/wk
RA45	RA	57 M	URI	1991	24.2	PSL 15	—
RA46	RA	76 M	URI	1643	53.5	PSL 3	MTX 4 mg/wk
Cont9	Control	31 F	URI	1331	NT	—	—
Cont10	Control	38 M	URI	478	NT	—	—
RA47	RA	71 F	Pleuritis	5581	162.4	PSL 4	Bucillamine 200 mg
RA-V2	RA with vasculitis	48 F	Sinusitis	1996	127.7	PSL 25	—
RA48	RA	76 F	UTI	11119	43.0	PSL 7.5	SASP 500 mg
RA49	RA	80 F	UTI	6138	85.7	PSL 5	MTX 6 mg
RA50	RA	74 F	UTI	2671	10.2	PSL 10	Bucillamine 200 mg
RA51	RA	63 F	Gingivitis	3539	49.7	PSL 5	MTX 5 mg
RA52	RA	82 F	Enteritis	4155	139.4	PSL 7	SASP 1000 mg, bucillamine 200 mg
RA-V3†	RA with vasculitis	56 F	Ileus	3437	115.1	PSL 5	Infliximab 3 mg/kg, MTX 6 mg
RA-V4†	RA with vasculitis	68 F	Cholecystitis	4725	1.9	mPSL 18	—
RA53	RA	79 F	Cholecystitis	2818	107.2	PSL 7	MTX 4 mg/wk
RA-V5†	RA with vasculitis	59 F	Cholecystitis	2571	83.3	PSL 7	MTX 4 mg/wk
Cont11	Control	57 M	Meningitis	1264	157.2	—	—
RA54	RA	67 F	Skin infection	2768	96.3	—	Leflunomide 10 mg
Cont12	Control	60 F	Phlegmone	8851	122.6	—	—
RA-V6	RA with vasculitis	70 F	Sepsis	14371	217.1	mPSL 6	MTX 8 mg
RA55	RA	70 F	Sepsis	8719	179.3	mPSlL 4	—

* Organizing pneumonia in RA27 was improved by the use of antibiotics, and not corticosteroids. Cholecystitis in RA-V4 and V5 and ileus in RA-V3 were a consequence of infection, and not of vasculitis. URI: upper respiratory infection, GST: gold sodium thiomalate, MTX: methotrexate, SASP: salazosulfapyridine, UTI: urinary tract infection.

infections caused by various pathogens, such as bacteria, viruses, fungi, and mycobacteria. To date, the utility of measuring CD64 for infection has been reported mainly for bacterial infection[20-22,24], but our data suggest that CD64 may be useful broadly as an infection marker for various kinds of pathogens. Interestingly, although the number of patients in the sample was small, we observed that the expression level of CD64 in tuberculosis was higher than that in infection by any other type of pathogen, although the difference was not statistically significant. Considering that concern about occurrence of infection, especially of tuberculosis, is increasing because of use of biologic agents for treatment of RA, CD64 can be a powerful, rapid, and easily accessible tool for screening of infection. A study reported that CD64 expression in gram-negative infections tended to be higher than in gram-positive infections[24]; however, our results showed no difference between them. The reason for this is unknown, but it may be because all our test samples were obtained before use of antibiotics; or it could be that all samples in the previous study were collected after starting treatment with antibiotics.

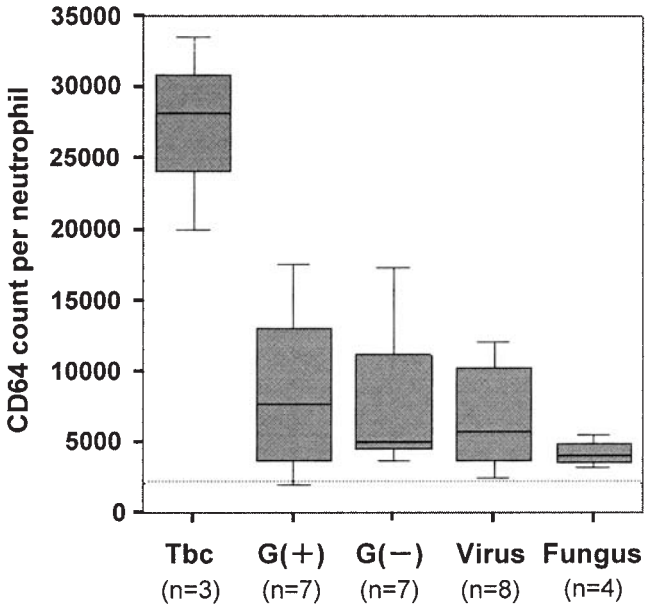

Figure 3. Comparison of CD64 expression on neutrophils among subjects infected with various types of pathogens. The line in the box indicates the median value; box shows 25th and 75th percentiles, and bars indicate 10th and 90th percentiles. Tbc: mycobacterium tuberculosis; G(+): gram-positive bacterium; G(–): gram-negative bacterium.

Our study showed that, in the absence of infection, the expression levels of CD64 in patients with RA were comparable with those in controls, and were not correlated with the titer of CRP. These results suggest that CD64 expression is not affected by the disease activity of RA itself. This supports the previous report that CD64 expression on peripheral blood neutrophils was within normal limits in patients with active RA[18].

However, our preliminary data showed that even in the absence of infection, CD64 expression was significantly increased in RA patients with vasculitis and in those with non-infectious active interstitial pneumonia. In patients with vasculitis, it has been reported that CD64 was upregulated because of the activation of neutrophils[24,26]. There have been no reports regarding CD64 expression in interstitial pneumonia to date. However, Garcia, *et al* suggested from analysis of bronchoalveolar lavage fluid that neutrophils in rheumatoid interstitial lung diseases were activated[27]. This may support our results. Although RA, vasculitis, interstitial pneumonia, and infection cause inflammation accompanied by elevation of inflammatory markers such as CRP or ESR, regulation of CD64 expression might be different among them. Their mechanisms are unknown; however, we speculate that different regulation might depend on cytokines that play a pivotal role in the respective diseases.

Inflammation is caused both by RA and by infection; therefore, they cannot be distinguished by inflammatory markers such as CRP or ESR. Our study showed that CD64 on neutrophils could distinguish inflammation caused by infection from that caused by RA itself. Therefore, CD64 could possibly be used as an infection marker in other inflammatory diseases. For CD64 to be useful as a marker for infection, its expression must be unaffected by the disease activity, as in RA. Szucs, *et al* have reported that CD64 expression of peripheral blood neutrophils in patients with systemic lupus erythematosus (SLE) was not upregulated; however, the activity of SLE at the time of measurement was not addressed[28]. In this regard, our preliminary data indicate that CD64 expression on neutrophils was upregulated in patients with active SLE. A similar phenomenon was observed in patients with active dermatomyositis. It may partially depend on the existence of interstitial pneumonia, which is often complicated in dermatomyositis. A recent study by Ureten, *et al* showed that

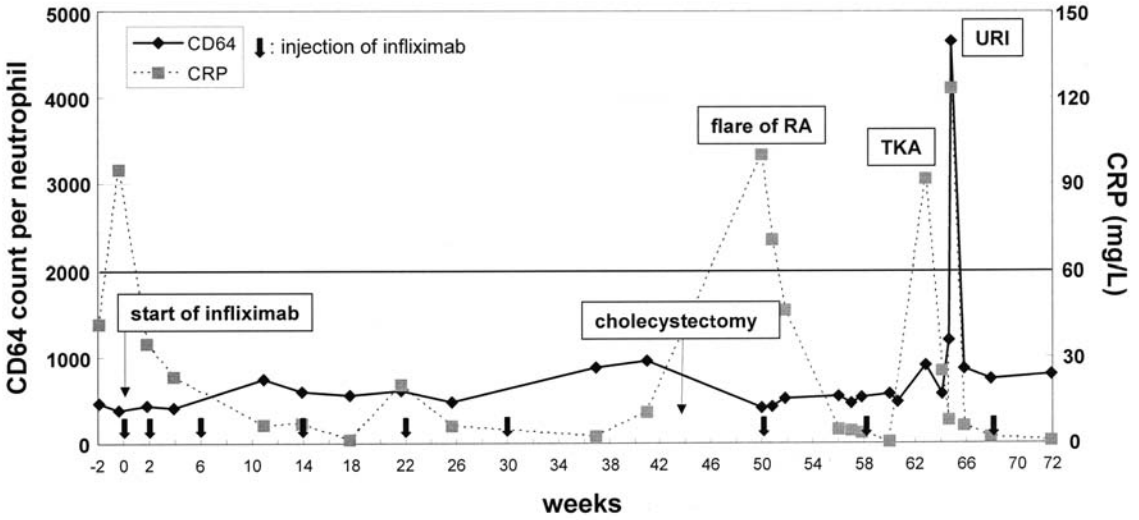

Figure 4. Longitudinal measurements of CD64, CRP, and clinical signs and symptoms in a representative patient with RA treated with infliximab, Patient S. TKA: total knee arthroplasty; URI: upper respiratory infection.

neutrophil CD64 expression was increased during exacerbation of Behçet's disease[29]. We speculate that the clinical utility of CD64 as an infection marker in inflammatory diseases other than RA might be limited. Further studies are needed to examine the usefulness of CD64 in other inflammatory diseases.

We have also confirmed the clinical utility of CD64 expression in RA regardless of the use of drugs, i.e., corticosteroids, DMARD, and also biologic agents. TNF-α is a key molecule in RA. TNF-α blockers such as etanercept, infliximab, and adalimumab are strong and effective agents for RA treatment, but there is concern that use of such agents might increase the incidence of opportunistic infection or tuberculosis. Although TNF-α is one of the inducers of CD64 expression on neutrophils, clinical data from our representative patient (Patient S) indicated not only that CD64 was useful for detection of infection but also that CD64 was not affected by the disease activity of RA, even during treatment with infliximab. A preliminary study showed that CD64 was also a useful marker in a patient with RA treated with anti-IL-6 receptor antibody (data not shown). We are planning studies with larger numbers of patients to establish the utility of CD64 during treatment with various biologic agents.

Additionally, clinical data from Patient S indicated that CD64 expression was unaffected by surgery such as total knee arthroplasty. This suggests that measurement of CD64 may be useful for detection of postoperative infection.

It should be kept in mind that there is limited clinical utility of CD64 in RA patients with vasculitis or interstitial pneumonia, although methods to detect infection are especially desirable in these cases. For treatment of vasculitis, we often use high-dose corticosteroids or immunosuppressants, which could induce immunosuppression in the patient. Interstitial pneumonia can occur in association with RA, but is also caused by drugs such as MTX or pathogens such as cytomegalovirus. Thus, differentiation of the cause of interstitial pneumonia is often clinically difficult. CD64 is useless alone in these conditions; however, it may have greater utility in combination with other laboratory markers. Further studies are needed.

Our study suggests that expression of CD64 on neutrophils is a highly sensitive and specific marker for detecting infection in RA and it can distinguish infection from an RA flare. The method to analyze expression of this surface marker is rapid and easily undertaken for diagnosis of infection. Further studies should address the identification of pathogens in combination with other surface molecules or the development of a simple method to rapidly detect expression of CD64 on neutrophils for the diagnosis of infection.

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