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# MODULATION OF MONOCYTE CHEMOTACTIC FUNCTION IN INFLAMMATORY LESIONS

## Role of Inflammatory Mediators[1]

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**Monocyte recruitment and accumulation in the synovial tissue is pivotal in the evolution of rheumatoid arthritis (RA). In the present study we examined the chemotactic potential of monocytes obtained from synovial fluid (SF) of patients with RA. Functionally, SF monocytes exhibited greatly diminished chemotactic activity to C5a compared with monocytes from the peripheral blood. In contrast, their chemotactic responsiveness to the synthetic peptide, FMLP, was nearly normal. To define a mechanism for this differential chemotactic dysfunction, cell-surface receptors for C5a (C5aR) and FMLP (FMLP-R) were evaluated. Whereas FMLP-R expression was similar on both blood and inflammatory monocytes, C5aR expression was markedly reduced on SF cells. Because decreased C5a binding in certain RA SF samples could not be attributed to free C5a, known or suspected components of inflammatory SF were evaluated for their ability to modulate chemotactic ligand receptors. Bacterial products including LPS and streptococcal cell walls, which are potent monocyte activators, down-regulated C5aR without affecting FMLP-R. Moreover, the cytokines IFN-γ and granulocyte-macrophage-CSF selectively decreased C5aR in parallel with decreased in vitro chemotactic activity to C5a. Thus, these data indicate that 1) synovial effusions may contain C5a and/or inflammatory mediators that modulate phenotypic and functional changes in monocytes, 2) chemotactic ligand receptors are independently regulated in inflammatory lesions, and 3) decreased C5aR expression and chemotactic potential likely provide a mechanism whereby monocyte-macrophages persist within the inflamed synovium.**

RA[3] is a chronic disease of unknown etiology in which inflammatory processes mediate synovial proliferation and erosive destruction of cartilage and bone. Extensive infiltration of monocyte-macrophages is thought to play an important role in the pathogenesis of this destructive joint disease (1). Monocytes obtained from inflammatory lesions are frequently activated and spontaneously secrete numerous inflammatory mediators, tissue destructive enzymes, and regulatory cytokines (1–4). Monocyte-derived cytokines including fibroblast growth factors, IL-1, TNF-α, CSF, and a mast cell growth factor (5–11) have been identified in the rheumatoid synovium. These monocyte products not only mediate other inflammatory cell activities, but also regulate the proliferation of synovial cells and promote erosion of cartilage and bone (12–14).

Based on the pivotal role monocytes play in the pathogenesis of RA, it is important to understand the factors that influence their traffic into the synovium. Mechanisms that are responsible for accumulation of monocytes into inflammatory lesions include chemotaxis as well as adherence and migration through synovial membranes. Adherence is controlled by monocyte surface molecules, such as Mac-1 (CDIIb) and p150,95 (CDIIc), which have been shown to be present in high levels on monocytes in the peripheral blood and the synovium in patients with RA (15, 16).

Directed migration, or chemotaxis, of monocytes is initiated by cell-surface receptor interaction with chemotactic ligands. Among the chemotactic ligand receptors on peripheral blood monocytes are those for the C cleavage fragment C5a and for the synthetic bacterial analog FMLP, which may be instrumental in monocyte recruitment to inflammatory and/or infectious sites (17, 18). The receptor-ligand interaction results in a calcium-dependent phosphoinositide hydrolysis and leads initially to chemotaxis (19). At higher concentrations, these chemotactic factors may promote further inflammation by inducing the release of inflammatory mediators (20–24).

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[3] Abbreviations used in this paper: RA, rheumatoid arthritis; CCE, counterflow centrifugal elutriation; C5aR, receptors for C5a; C5aR$^+$, cells that express C5aR; FMF, flow microfluorometry; FMLP-R, receptors for FMLP; FMLP-R$^+$, cells that express FMLP-R; GM-CSF, granulocyte-macrophage CSF; SCW, streptococcal cell wall; SF, synovial fluid; SFMC, synovial fluid mononuclear cells; FcγR$^+$, cells that express FcγR; TR, Texas red; FMLPL, FMLP-lysine; DmEm, Dulbecco's modified Eagle's medium.

In the present study, we evaluated monocytes derived from an inflammatory site for their chemotactic phenotype and function. In contrast to blood monocytes, the majority of monocytes from the SF of RA patients have few receptors for C5a, but have normal numbers of FMLP-R, and furthermore these cells exhibit a corresponding decreased chemotaxis in response to C5a, but not to FMLP. This selective phenotypic and functional modulation of inflammatory cells could be mimicked after exposure of blood monocytes to inflammatory products in vitro. The inverse correlation between activation and migration may provide a mechanism for accumulation of mononuclear cells within an inflammatory lesion.

## MATERIALS AND METHODS

*SF collection and isolation of mononuclear cells.* SF from clinically tender and swollen knees of adult patients with confirmed definite or classic RA was aspirated under aseptic conditions with a 20-gauge needle into a sterile syringe. The fluid was maintained in the syringe at room temperature and was processed within 3 h. The fluid was centrifuged and the cell pellet was resuspended in PBS. These cells were then centrifuged on Ficoll-Paque gradients (25) and the SFMC were collected from the interface, washed and resuspended in DmEm containing 50 μg/ml gentamicin and 2 mM glutamine. The remaining cellfree SF was frozen at −20°C until assayed.

*Peripheral blood mononuclear leukocytes.* Peripheral blood was obtained from adult RA patients or from normal volunteers, diluted in PBS, and layered on Ficoll-Paque gradients. After centrifugation, the interface containing PBMC was collected, washed, and resuspended in DmEm. To obtain highly purified populations of monocytes, the PBMC were separated by CCE and were characterized as described (26, 27).

*Monocyte culture with SF, cytokines, and bacterial products.* CCE-purified monocytes ($5 \times 10^6$/ml) were incubated in suspension for 1 or 2 h in serum-free DmEm only (control), or with the cellfree portion of SF from RA patients at 37°C or 4°C. Additionally, monocytes were suspended in DMEM for 2 to 48 h in the presence or absence of indicated concentrations of IFN-γ, GM-CSF, or TNF-α (Genzyme, Boston, MA) at 37°C. Bacterial products including LPS (*Escherichia coli* 055:B5; Difco, Detroit, MI) or SCW preparations prepared from group A streptococci (28) were added to monocytes in suspension for 12 to 48 h at 37°C. Monocytes were then washed, stained for FMF analysis, or assayed for chemotactic activity.

*Fluorescence-binding studies.* SFMC and PBMC or CCE-purified monocytes ($5 \times 10^5$/50 μl) were incubated in PBS with 0.1% sodium azide containing saturating concentrations (determined for each preparation in preliminary experiments) of FITC-conjugated C5a, TR-FMLPL, both reagents simultaneously, or with FITC-FMLPL (Peninsula Laboratories, Belmont, CA) for 30 min at 4°C (17). For determination of background fluorescence, control cells were incubated with unconjugated FITC and/or TR in concentrations similar to those present in FITC-C5a or TR-FMLPL. In some experiments, unstained cells were used as controls because the fluorescence histograms of unstained cells were similar to those incubated with unconjugated TR or FITC (17). Cells were then washed twice with 1 ml of PBS containing 0.1% sodium azide at 4°C and were immediately resuspended in 0.5 ml 2% paraformaldehyde to prevent dissociation of FMLPL and/or C5a from their receptors. Cells were maintained at 4°C until analysis by FMF.

*Dual labeling for surface Ag and chemotactic receptors.* Single cell suspensions (50 μl) of SFMC or PBMC ($1 \times 10^7$/ml in PBS with 0.1% sodium azide) were first incubated at 4°C for 30 min with rabbit IgG anti-DNP dimers (kindly provided by Dr. Paul Plotz, National Institute of Arthritis, Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, MD), washed, and then incubated with FITC-labeled $F(ab')_2$ goat anti-rabbit IgG (Tago, Burlington, CA). After staining, the cells were washed and exposed to saturating concentrations of TR-FMLPL. The cells were then washed twice, immediately resuspended in 0.5 ml 2% paraformaldehyde, and maintained at 4°C until analysis by dual parameter FMF.

*Dual parameter FMF.* Data from single parameter ($1 \times 10^4$ cells) or dual parameter ($2.5 \times 10^4$ cells) FMF were collected either for red or green fluorescence or simultaneously for red and green fluorescence with a dual laser FACS II or FACStar (Becton Dickinson FACS Systems, Sunnyvale, CA) as described (17).

*Monocyte chemotaxis.* SFMC, PBMC, or CCE-purified monocytes were assayed routinely at $1.1 \times 10^6$ monocytes/ml in Gey's balanced salt solution (National Institutes of Health Media Unit) containing 2% BSA, 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.015 M HEPES. The chemotaxis assay was carried out in 48-well microchamber plates (Neuroprobe, Rockville, MD) by using FMLP (Peninsula Laboratories) and C5a des Arg (derived from endotoxin-activated serum) (17, 27). After a 90-min incubation at 37°C, the polycarbonate filters were removed, fixed, and stained with Diff-Quik (American Scientific Products, Columbia, MD) for quantitation with an Optomax Image Analyzer (Optomax, Hollis, NH). Chemotactic activity is defined as the mean (±SEM) number of monocytes that migrated through the filter pores in three standard fields for each of triplicate filters.

## RESULTS

*Chemotactic function of synovial and blood monocytes.* Mononuclear cells obtained from the SF of eight patients with RA were exposed to different concentrations of C5a and FMLP in an in vitro chemotaxis assay. In each experiment, chemotaxis of PBMC from healthy donors was determined simultaneously. Synovial monocytes from all patients tested exhibited markedly lower chemotactic responses to C5a than peripheral blood monocytes. Decreased responsiveness was apparent and significantly different at all C5a concentrations tested (Fig. 1*A*). In contrast, chemotactic responses of SFMC to FMLP were not significantly different from PBMC (Fig. 1*B*) and were occasionally higher.

To determine whether RA patients have a generalized monocyte chemotactic defect, the ability of monocytes from both SF and peripheral blood of the same RA patients to migrate to C5a and FMLP was compared simultaneously. Chemotaxis of SFMC to C5a was again diminished compared with PBMC from the same patients, whereas chemotaxis to FMLP was similar in both populations (data not shown). Furthermore, PBMC from RA patients and healthy subjects migrated at similar levels. It thus appears that the SFMC, but not PBMC, from patients with RA are selectively deficient in their ability to respond to C5a.

*Expression of chemotactic ligand receptors on SFMC and PBMC.* To determine whether the deficient chemotactic response to C5a was related to altered expression of chemotactic ligand receptors on the SFMC, subsequent experiments evaluated C5aR and FMLP-R on these cells by dual parameter FMF. SFMC and PBMC were incubated with saturating concentrations of IgG dimers (FITC) to label monocytes followed by saturation with fluoro-

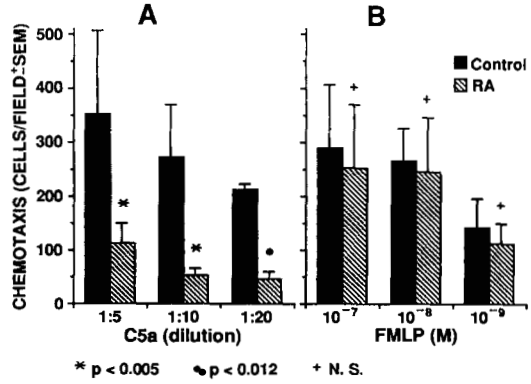


*Figure 1.* SFMC and PBMC chemotaxis to C5a and FMLP. RA SFMC from eight patients and control PBMC from eight normal volunteers were assayed for chemotactic activity in response to the indicated concentrations of C5a des Arg (*A*), FMLP (*B*), or buffer only (0). Values represent the arithmetic mean ± SEM of monocytes that migrated through the 5-μm filter pores in three standard fields for each of triplicate filters from the eight experiments. Probability value was calculated by using Student's *t*-test.

chrome-labeled FMLPL (TR) for analysis by FMF. Dual parameter FACS profiles indicated that almost all monocytes (FcγR$^+$ dull cells) were also FMLP-R$^+$ (Fig. 2*A* and *B*, cells in box labeled *1*) in both SFMC and PBMC. It is also of interest to note that FcγR$^+$ bright cells, which represent NK cells and have been previously shown to bind C5a (29) (Fig. 2*B*, cells in box labeled 2), were absent from this and all other SF populations we analyzed.

Because essentially all of the SFMC and PBMC were FMLP-R$^+$ (17), we next evaluated C5aR on the FMLP-R$^+$ cells in these populations by dual color fluorescence. FMLP-R$^+$ cells represented 62% of the SFMC (Fig. 3*A* (box labeled *1*) and Fig. 3*D*). In marked contrast, C5aR$^+$ cells stained at such low levels that there was only a marginal shift in the fluorescence profile with no clear indication of a C5aR$^+$ cell population (Fig. 3*A*, box labeled 2). In the single fluorescence profiles, there was only minimal fluorescence above background control levels (Fig. 3*C*). By using a computer subtraction program, C5aR$^+$ cells were calculated to represent 7.3% of the SFMC. These findings indicate that a large subpopulation (87% in this particular sample) of the FMLP-R$^+$ SFMC do not bind C5a.

*C5a-dependent and -independent modulation of C5aR.* Because normal and inflammatory monocytes can synthesize and secrete C5a (30), and SF has been shown to contain C5a (31), we examined whether SF from patients with RA could modulate C5aR expression via a decrease in the available receptor sites for C5a binding, through internalization of C5a-C5aR complexes, or through receptor down-regulation. CCE-purified monocytes were incubated in media with or without 20% SF at 37°C for only 2 h, washed, saturated with FITC-C5a, and analyzed by FMF. Monocytes preincubated with SF

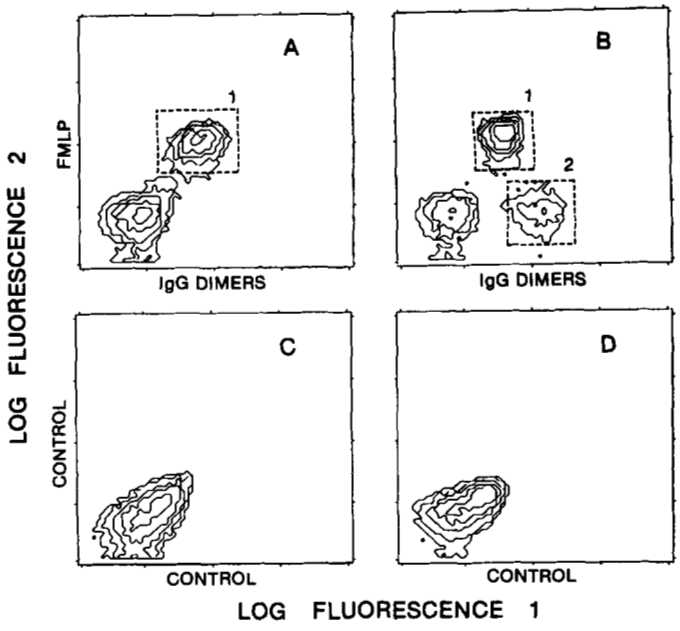


*Figure 2.* SFMC and PBMC expression of FMLP-R. SFMC (*A* and *C*) and PBMC (*B* and *D*) were saturated with rabbit IgG anti-DNP dimers followed by FITC-labeled F(ab′)$_2$ goat anti-rabbit Ig to label FcγR. The cells were then incubated with saturating concentrations of TR-FMLPL (*A* and *B*). For controls, the cells (*C* and *D*) were first incubated with DmEm alone followed by incubation with FITC-labeled F(ab′)$_2$ goat anti-rabbit IgG. Cells were fixed in 2% paraformaldehyde and were analyzed by dual parameter FMF with the use of logarithmic amplification. In *A* and *B*, cells in the box labeled *1* represent the monocyte populations (FcγR$^+$ dull) that express FMLP-R. In *B*, cells within the box labeled *2* represent the NK cell population (FcγR$^+$ bright) that do not bear FMLP-R.

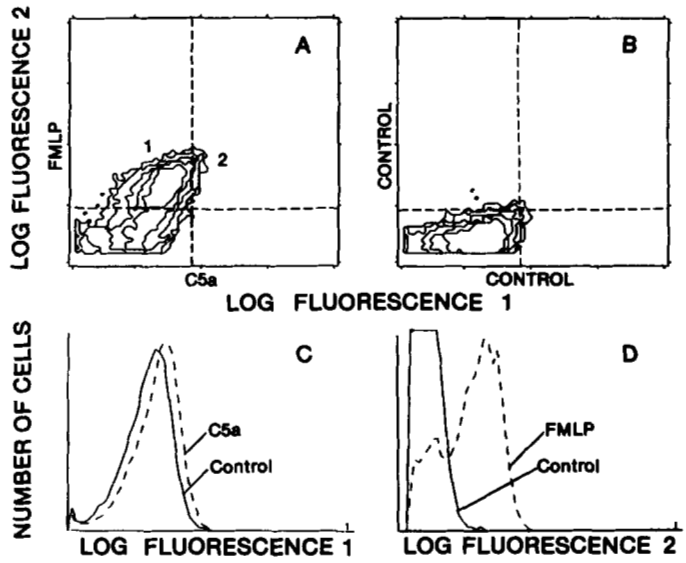


*Figure 3.* Coexpression of C5aR and FMLP-R on SFMC. SFMC were simultaneously incubated with saturating concentrations of FITC-C5a and TR-FMLPL (*A*) or FITC and TR alone (*B*), washed immediately, and fixed in 2% paraformaldehyde. Cells were analyzed by dual parameter FMF by using logarithmic amplification. *A* and *B* represent dual fluorescence cell-sorter profiles. *C* and *D* represent the single fluorescence profiles that were obtained from the dual fluorescence cell-sorter data with the help of a special computer program, for FITC or TR alone control (*solid lines*) and for FITC-C5a or TR-FMLPL (*dotted lines*).

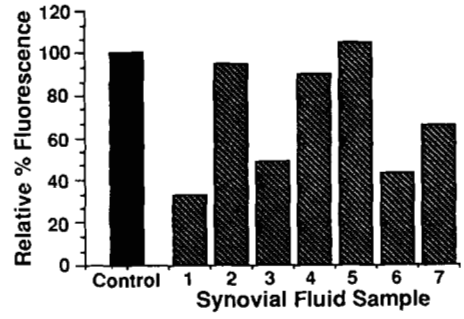


*Figure 4.* Preincubation with some, but not all, SF leads to a decrease in C5a binding. CCE-purified monocytes were incubated with media (control) or with 20% SF for 1 h (samples 3 to 7) or 2 h (samples 1, 2), washed, saturated with FITC-C5a, washed again, and analyzed by FMF with the use of linear amplification. Fluorescence of control monocytes represents 100%. Samples incubated with SF were compared with the control sample, and the data expressed as relative % fluorescence.

bound 65% less FITC-C5a as measured by total fluorescence (data not shown). These results indicate a decreased C5a-binding capacity, but do not differentiate between C5aR down-regulation vs receptor occupancy by C5a. Therefore, CCE-purified monocytes were incubated with 0 to 40% SF for 2 h at 4°C in the presence of 0.1% sodium azide to prevent C5aR modulation. Incubation with 10 or 20% SF decreased binding of FITC-C5a by 58% and 72%, respectively, with no further decrease at 40% SF concentration (data not shown). Subsequently, of seven SF samples studied, only four caused variable levels of inhibition of C5a binding to the monocytes (Fig. 4). Thus, although the decreased C5a binding in certain SF may be attributed to free C5a, additional mechanisms are likely present that can modulate expression of these receptors on inflammatory monocytes.

*Modulation of monocyte C5aR by bacterial products.* To determine the role of factors other than C5a on monocyte chemotactic ability, the impact of other known or

suspected products found in SF that might modulate chemotactic ligand receptor expression was evaluated.

Because bacterial products contribute to many inflammatory lesions, induce inflammatory arthritis in animal models, and stimulate numerous monocyte functions (32–36), we first examined the effect of SCW from Gram-positive bacteria and LPS from Gram-negative organisms on monocyte chemotactic responsiveness. CCE-purified monocytes were incubated for 12 to 36 h with LPS and evaluated for receptor levels by FMF. Exposure of monocytes to LPS resulted in a 69% decrease in $C5aR^+$ cells by 12 h and 84% by 36 h, indicating that many of the monocytes became unable to bind C5a (Fig. 5*A*). In addition, at each time point, the density of C5aR (measured by the median fluorescence intensity) on the remaining $C5aR^+$ cells was ≥40% lower than on control monocytes (data not shown). Similar evaluation of FMLP-R revealed that whereas LPS induced a transient decrease in FMLP-R at 12 h, an increase in FMLP-R expression was apparent at 24 to 36 h (Fig. 5*A*). Not only LPS, but also SCW treatment of monocytes (data not shown) independently regulated C5aR and FMLP-R expression. A decreased chemotactic response to C5a and normal chemotactic response to FMLP was displayed by monocytes incubated with LPS (Fig. 5*B* and *C*).

*IFN-γ modulation of chemotactic receptor expression and function.* In addition to potential bacterial products, numerous inflammatory cytokines have been shown to be released locally by synovial lymphocytes and monocytes (5–11). To determine whether such locally produced immunoregulatory products might influence monocyte function, we evaluated several relevant purified and recombinant cytokines for their influence on chemotaxis. IFN-γ, a potent modulator of monocyte phenotype and function (37), was added to monocytes that were then monitored for chemotactic function and associated receptor expression. As shown in Figure 6, incubation of monocytes with IFN-γ (500 U/ml) induced a marked increase in expression of the MHC class II Ag, HLA-DR, one of its known functions (38). However, associated with the increase in HLA-DR was a marked decrease in the levels of C5aR on the same population of cells (Fig. 6). Thus, there appeared to be an inverse correlation between monocyte activation/differentiation as measured by HLA-DR expression and the number of C5aR on individual cells. Furthermore, the decrease in C5aR levels was clearly associated with diminished ability of these cells to respond in a positive fashion to C5a in the chemotaxis assay (Fig. 7).

*Cytokine modulation of monocyte chemotaxis.* Although IFN-γ clearly influenced monocyte chemotactic ligand receptor levels and function, recent evidence suggests that IFN-γ may not be a relevant mediator in the events leading to RA (1, 39). More recently, GM-CSF has been implicated as the primary macrophage-activating factor in these lesions (11). We therefore evaluated GM-CSF, as well as TNF-α, which stimulates monocytes (40) and has been identified in SF (9, 10), for their effects on monocyte chemotaxis. Similar to IFN-γ, GM-CSF markedly inhibited C5aR receptor expression while augmenting HLA-DR Ag, whereas TNF-α minimally altered C5aR or HLA-DR levels (Fig. 6). FMLP-R levels remained stable independent of these cytokines (data not shown). Consistent with the phenotypic observations, monocytes

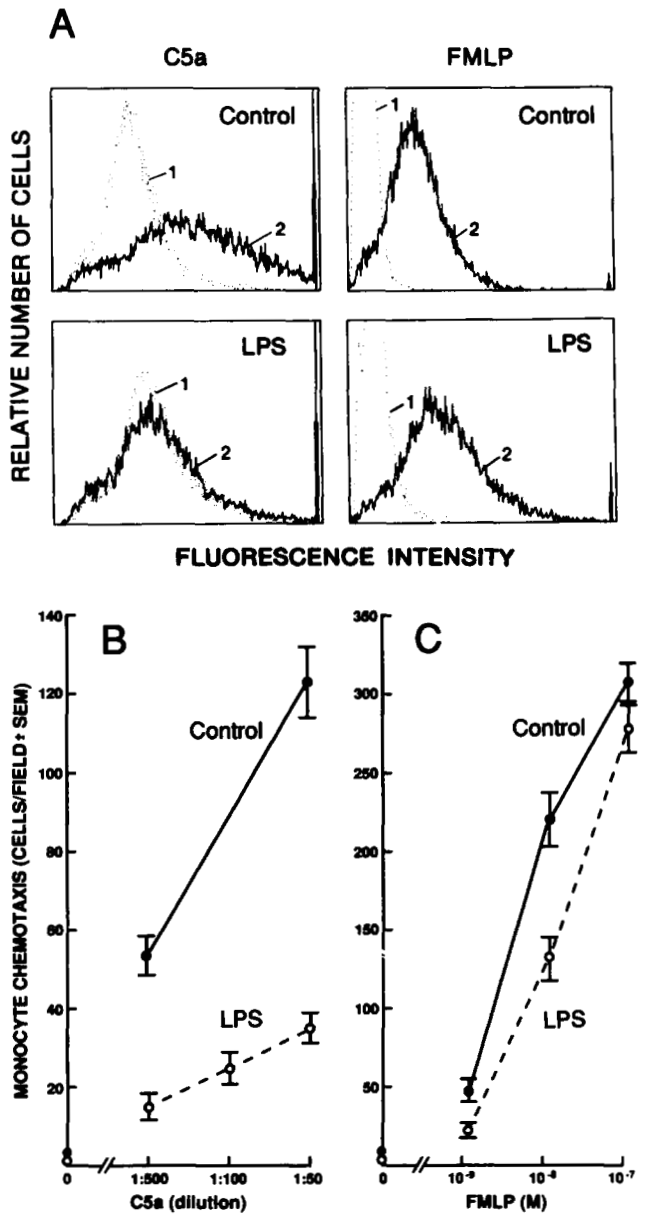


*Figure 5.* Monocyte activation by LPS inhibits C5aR expression and function. CCE-purified monocytes were incubated with 10 μg LPS/ml or with DmEm only (control) for 36 h. A. The LPS-treated and control cells were washed, saturated with FITC-C5a or FITC-FMLPL (fluorescence profiles labeled with 2) or with no ligand (fluorescence profiles labeled with 1) and analyzed by FMF with the use of linear amplification. *B* and *C*, monocytes were incubated with 10 μg LPS/ml (*dotted lines*) or with media alone (*solid lines*) for 24 h. Cells were washed and assayed for chemotactic activity toward the indicated concentrations of C5a (*B*), FMLP (*C*), or buffer only (0). Values represent the mean number of monocytes that migrated through the 5-μm filter pores in three standard fields for each of triplicate filters.

treated with GM-CSF exhibited a marked decrease in chemotaxis to C5a at all concentrations tested (Fig. 8). In numerous experiments, however, TNF-α did not inhibit and sometimes enhanced (as shown in Fig. 8) monocyte migration to C5a. Thus, exposure of monocytes to certain inflammatory stimuli that augment HLA-DR induces a decrease in receptors for C5a, effectively impairing the ability of these cells to functionally respond to this chemotactic stimulus.

To examine whether lymphokine activation leads to C5aR down-regulation via C5a production and binding, supernatants from monocytes cultured 24 h in media

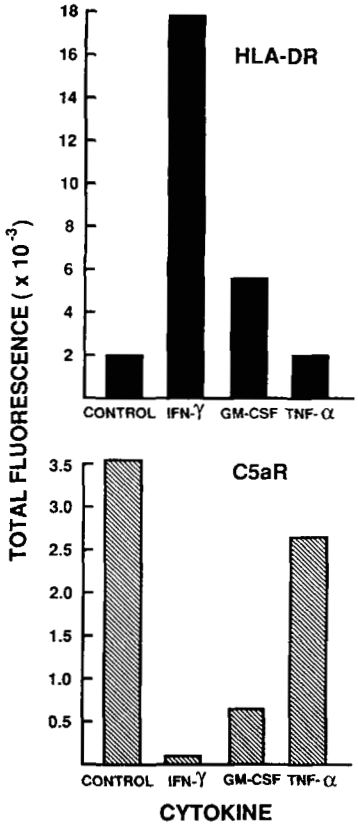


*Figure 6.* Effect of cytokines on HLA-DR Ag and C5aR expression. Monocytes were incubated with media (control), IFN-γ (500 U/ml), GM-CSF (500 U/ml) or TNF-α ($2 \times 10^{-10}$ M) for 36 h. Cells were then incubated with saturating concentrations of FITC-HLA-DR, FITC-C5a, or control antibodies and were analyzed by FMF by using linear amplification. Total fluorescence was calculated as percent positive cells × relative median fluorescence intensity (median fluorescence intensity − background fluorescence).

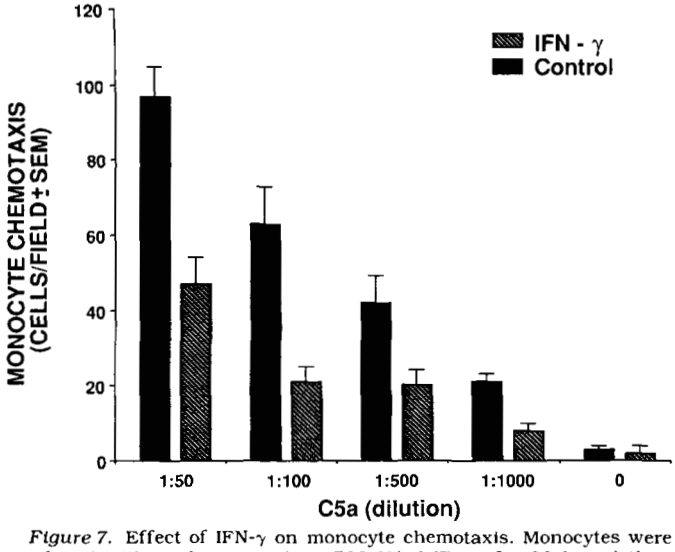


*Figure 7.* Effect of IFN-γ on monocyte chemotaxis. Monocytes were incubated with media (control) or 500 U/ml IFN-γ for 36 h and their chemotactic activity to C5a determined as in Figure 1.

only (control) or media containing IFN-γ or TNF-α were tested for their ability to alter subsequent binding of FITC-C5a. Because no decrease in FITC-C5a binding was observed when monocytes were preincubated for 1 h at 4°C with the IFN-γ- or TNF-α-stimulated monocyte culture supernatants as compared with unstimulated monocyte culture supernatants (data not shown), the mechanism of lymphokine-induced C5aR modulation is likely independent of C5a production and binding.

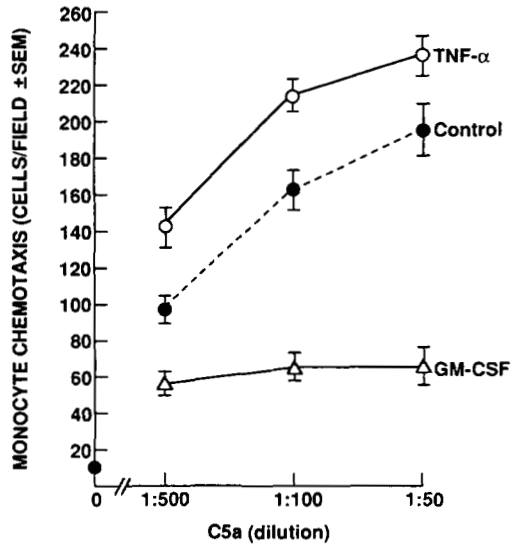


*Figure 8.* Effect of GM-CSF and TNF-α on monocyte chemotaxis to C5a. Monocytes were incubated with media (control), GM-CSF (500 U/ml), or TNF-α ($2 \times 10^{-10}$ M) for 36 h. Chemotaxis to the indicated concentrations of C5a or buffer only (0) was determined as in Figure 1.

## DISCUSSION

SF monocytes from patients with RA exhibit a marked dysfunctional response to the chemotactic ligand C5a. This abnormality was specific to monocytes isolated from the inflammatory site and did not represent a generalized chemotactic defect because peripheral blood monocytes from the same patients responded normally to C5a. Moreover, this deficient SF monocyte chemotactic response was selective for C5a in that FMLP responsiveness was not impaired in these inflammatory cell populations.

In defining potential mechanisms for this selective impairment of migratory function, we demonstrated that receptors for C5a on the synovial monocyte cell surface were markedly reduced compared with peripheral blood monocytes, rendering them less susceptible to C5a. On the other hand, FMLP-R were only minimally altered, if at all, on these same populations. A similar dichotomy of C5aR and FMLP-R expression has been reported on peripheral blood monocytes from AIDS patients (41). Moreover, differential regulation of chemotactic receptors is not unique to monocytes, because down-regulation of C5aR and concomitant up-regulation of FMLP-R also occurs after activation of human neutrophils (42). Although the underlying mechanism for this differential receptor regulation is unknown, monocytes with a low C5aR phenotype are clearly present in the SF of patients with RA. Because such a subpopulation of monocytes is not apparent in the peripheral blood of these patients, this abnormality is most likely acquired because of the influence of local factors.

Consistent with this hypothesis was our finding that peripheral blood monocytes from healthy individuals also lost some C5a-binding capacity during a 2-h incubation with SF from RA patients. One possible explanation for this finding is that interaction of C5a in the inflammatory fluid (30) with the C5aR on SFMC leads to receptor oc-

cupancy and/or induces internalization of the receptor-ligand complex. Indeed, we have also demonstrated that SF from some patients with RA may contain C5a. In addition, SF may contain factors other than C5a that modulate C5aR expression. In this regard, we initially evaluated selected bacterial cell products, which may function as potential eliciting agents in certain types of inflammatory arthritis (32), for their effects on monocyte chemotaxis. LPS, a potent macrophage-activating agent (33), and group A SCW components that activate monocytes (34) and induce arthritis in animals (35), caused a dramatic decrease in C5aR expression and chemotactic responsiveness to C5a. The majority of peripheral blood monocytes lost their C5aR entirely in the presence of these bacterial constituents and the remaining cells showed decreased receptor density. These changes were detectable within 12 h after exposure to the bacterial products and persisted during the following 24 to 48 h in culture.

Inflammatory SF also contains cytokines that may contribute to the regulation of monocyte chemotaxis. Although IFN-$\gamma$ was a potent modulator of monocyte C5aR and chemotactic function, IFN-$\gamma$ levels are minimal or not detectable in SF (39). Therefore, we evaluated TNF-$\alpha$ and GM-CSF, two cytokines emerging as important contributors to the pathogenic mechanisms of RA (1), for their influence on monocyte chemotaxis. Whereas GM-CSF up-regulated HLA-DR as previously demonstrated (43) and down-regulated C5aR, TNF-$\alpha$ had little effect on either HLA-DR or C5aR. Thus, there appears to be a correlation between the ability of an inflammatory stimulus to induce monocyte differentiation as monitored by HLA-DR levels and its ability to inhibit the expression of C5aR. Moreover, activation and/or maturation of monocytes by a variety of stimuli within an inflammatory site may induce the release of GM-CSF, resulting in an autocrine loop whereby monocytes inhibit their own migratory potential to C5a and become immobilized. Additionally, increased receptor responsiveness to inflammatory stimuli (44) and increased expression of adhesion molecules (45) occur in activated monocytes. By these mechanisms, large numbers of activated monocyte-macrophages may persist in chronic infectious or inflammatory sites where they mediate the inflammatory process and/or tissue destruction characteristic of RA.

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