Different monocyte reaction patterns in newly diagnosed, untreated rheumatoid arthritis and lupus patients probably confer disparate C-reactive protein levels

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Abstract

Objectives

To investigate the different capacities of monocytes to produce cytokines in newly diagnosed, untreated patients with rheumatoid arthritis (RA) or systemic lupus and to examine the possible correlation among serum C-reactive protein (CRP), cytokines, swollen joint counts, and erythrocyte sedimentation rates (ESR) in untreated RA patients.

Methods

Monocytes from untreated RA or lupus patients were cultured in vitro *with lipopolysaccharide (LPS, as bacterial infection) or immune complexes (as endogenous immune deviation) and supernatants were collected for cytokine determination. Sera from RA patients were assayed for interleukin-6 (IL-6), IL-1*β*, IL-10, tumor necrosis factor-alpha (TNF-*α*) and IL-1 receptor antagonist (IL-1ra). These cytokines were related to serum CRP, swollen joint counts, and ESR.*

Results

*RA monocytes uniformly produced IL-6, IL-1*β*, TNF-*α*, or IL-10* in vitro. *In contrast, lupus monocytes could be divided into two subsets: (i) monocytes which produce cytokines on LPS stimulation but not on challenging with immune complexes; and (ii) monocytes which, interestingly, generate cytokines on stimulation by immune complexes but not LPS. These cytokines in turn stimulate the liver to synthesize CRP differently in the SLE subsets and RA patients. Moreover, serum IL-1ra levels correlated significantly with serum IL-6, IL-1*β*, and TNF-*α* concentrations (p = 0.005, 0.008, or 0.040, respectively), but not with IL-10 (p = 0.582) in RA patients.*

Conclusions

*Two lupus subsets exist that react either to LPS or immune complexes to produce CRP-inducing cytokines, in contrast to homogeneous RA monocytes. This is the first report that different reaction patterns of CRP-inducing cytokine production in RA and lupus monocytes probably underlie the high CRP levels in RA versus low heterogeneity in lupus. The correlation of serum IL-1ra levels with serum IL-6, IL-1*β*, or TNF-*α* concentrations, and the borderline correlation of the former with CRP levels, demonstrate that IL-1ra is an acute phase reactant in RA as well as in SLE patients.*

Key words

Untreated rheumatoid arthritis and untreated lupus, monocytes, *in vitro* cytokines, C reactive protein.

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Introduction

While C-reactive protein (CRP) and the erythrocyte sedimentation rate (ESR) have been used to assess RA disease activity (1), lupus patients typically have low or near normal CRP in their sera (2, 3). Although SLE sera have much lower CRP-inducing cytokines than RA sera (4), there is still no answer pointing to a particular cell type to explain this phenomenon after many years of research. Blood monocytes and tissue macrophages, which synthesize all three CRP-inducing cytokines (5), are intimately involved in initiating an acute phase response. Only monocytes/macrophages can simultaneously generate the three CRP-inducing cytokines (IL-6, IL-1, and TNF-α), together with IL-10 and IL-1ra (5-7). Thus,investigating the *in vitro* production of these monokines from untreated RA and lupus patients should help to clarify the mechanism behind high serum CRP and CRP-inducing cytokine levels, in contrast to lupus levels, in RA patients (4). Whether this presumed diversity of secreted monokines makes any difference in the relationship between the five above mentioned cytokines and CRP in newly diagnosed RA patients compared to untreated lupus patients (4) is also interesting and has not been studied before. It is well known that hepatocytes respond to IL-6, IL-1, and TNF-α produced after infection, injury, or tissue trauma to synthesize CRP (8, 9). Moreover, CRP has long been used as a laboratory parameter for acute phase response of RA since suppression of elevated CRP in active RA confers functional improvement (10). While serum IL-6 levels were found to correlate with CRP in RA patients (11), these patients were either receiving disease-modifying anti-rheumatic drugs (DMARDs) or were under prednisolone treatment (12,13) during the time of investigation. Moreover, serum IL-10 levels were also elevated in treated RA patients (11). However, up to now, no published reports have studied three key CRP-inducing cytokines, IL-10, IL-1ra (14), as well as CRP together in the same sera from newly diagnosed untreated RA patients. Since serum CRP levels have been shown not to correlate individually with IL-6, IL-1, or TNF-α in untreated lupus patients (4), whether a dissimilar phenomenon in untreated RA patients from those treated (11) remains unknown. Accordingly, these three CRP-inducing cytokines, IL-10, IL-1ra and CRP together, were investigated in the same sera from newly diagnosed, untreated patients (see Materials and Methods) to determine the true relationship between serum cytokines and CRP in RA.

Materials and methods

Recruitment of patients, and controls

Patients who fulfilled the 1987 American College of Rheumatology (ACR) criteria for rheumatoid arthritis, and the 1997 revised ACR criteria for systemic lupus erythematosus (SLE), were enrolled in this study. RA patients were included as a disease control for SLE. Serum samples from 25 RA patients, 26 SLE patients and 14 healthy controls (see below for inclusion and exclusion criteria) were collected. The demographic, clinical and laboratory data are shown in Table I. Since 4 SLE patients for *in vitro* study were from reference 4 and others were later enrolled, all data were considered together for lupus patients in Table I. These hospitalized RA and lupus patients were enrolled consecutively. Only newly diagnosed, untreated patients were included to avoid possible manipulation of the disease process, except that non-steroidal anti-inflammatory drugs (NSAIDs) were allowed for ethical reasons. Moreover, cyclooxygenase-2 inhibition does not have T-cell immunomodulatory effects (15). The healthy controls had no history of chronic hepatitis, sinusitis, allergic diseases (such as asthma or rhinitis) or acute infections. All subjects gave their informed consent before blood collection.

Collection of serum samples and reagents

Sera were obtained and stored as reported previously (4). Antibodies and recombinant cytokines for ELISA assay were the same as described (4). Similarly, polyclonal goat and monoclonal mouse anti-IL-10 with recombinant IL-10 were purchased from R&D

Table I. Demographic, clinical and laboratory data of enrolled patients.

	SLE	RA
Number of patients	26	25
Sex (M/F)	5/21	5/20
Age	32.1 (16-58)	44.5 (19-77)
Disease duration from initial symptoms	25 days	5 months
median and range	4 days-48 months	1-48.5 months
ESR (mm/h)	61.8 (15-141)	76.4 (15-155)
Rheumatoid factor (< 40 IU/ml)		374 (126-1320)
Anti-dsDNA antibody (< 1:10)	1:464 (0-1:1280)	
Swollen joint count		10.7 (4-27)
Proteinuria > 0.5 g/day	7/26	0/22

SLE: systemic lupus erythematosus; RA: rheumatoid arthritis; ESR: erythrocyte sedimentation rate. Data are presented as means (range) except where indicated.

(Minneapolis, MN, USA). All these antibodies are neutralizing antibodies, which should detect cytokines with active forms. Human serum albumin (HSA), rabbit polyclonal IgG anti-HSA antibodies (anti-HSA) and lipopolysaccharide (LPS) were obtained from Sigma (St. Louis, USA); control rabbit IgG (Control IgG) was purchased from Jackson ImmunoResearch (West Grove, PA, USA). Fetal calf serum (FCS) was obtained from Hyclone (Logan, UT, USA) and treated at 56ºC for 30 minutes before use. RPMI-1640 medium and Hanks balanced salt solution (HBSS) were purchased from Gibco (Grand Island, NY, USA).

CRP and cytokine assay

Serum CRP levels were determined by nephelometry and those less than the detection sensitivity of 5 mg/L were assigned arbitrarily the value 0 mg/L for easy comparison. IL-6, IL-1β, IL-10, TNF-α and IL-1ra in the sera and culture supernatants were measured by ELISA using specific anti-cytokine antibodies. The procedures employed here were the same as described (4), except that anti-IL-10 antibody was also used in ELISA plates. Sera and culture supernatants were added at four-fold dilutions and results were multiplied by four to determine the original concentrations. According to the manufacturer, no cross-reactivity between these products and other cytokines had occurred. The assay sensitivity for IL-6, IL-1β, IL-10, TNF-α, and IL-1ra was 10 to 20 pg/ml, 5 pg/ml, 100 to 200 pg/ml, 2 pg/ml, and 10 pg/ml, respectively.

Since detailed studies on the production of CRP by human hepatocytes after stimulation with IL-1β, IL-6, or TNF-α have been published in the past 13 years (8,9,16), there was no attempt in this study to examine the ability of lupus hepatocytes to produce CRP by various cytokines, compared with RA hepatocytes. Hence, CRP-inducing cytokine (IL-1β, IL-6, or TNF-α) patterns from the main secretor (monocytes, secreting all three cytokines together) of RA or SLE are used indirectly to relate to different CRP levels in these two autoimmune diseases. This kind of monocyte reaction patterns is mimicking the *in vivo* situation by using both LPS (representing a form of bacterial infection) and immune complexes (representing endogenous immune deviation).

Separation of blood cell components

Heparinized blood was processed and the same media as in (4) were used. The purity of monocytes was at least 90% in the adherent cell fraction when determined by non-specific esterase and flow cytometric CD14 staining. Fewer than 5% of monocytes were detected in the non-adherent lymphocyte portion by the non-specific esterase criterion and Wright stain examination.

Immune complex generation and in vitro *monocyte culture*

Immune complexes were produced as described (4). Monocytes in RPMI-1640 with 2% FCS at 1 to 2 x 10^5 per well in duplicate were cultured in immune complex-prepared plates with appropriate LPS concentrations (3 or 10 mg/ml) or HSA/anti-HSA complex amounts (30 or 60 mg) for two and 12 days as described (4). Final results were adjusted for 2 x 10^5 cells. The culture supernatants were then stored at -20ºC until cytokine amounts were assayed, and mean values ± standard deviation (S.D.) were obtained. For accumulated *in vitro* results, the data are expressed as a mean of all means.

Statistical analysis

The significant p level of 0.05 for *in vitro* results was determined by the unpaired t-test on Macintosh Staview II software. The correlations between CRP or cytokines and the swollen joint count are presented as X-Y linear correlation pictures. Macintosh Staview II software was used to analyze all the statistical data regarding linear correlation. All p values of individual correlation coefficients (r) obtained from Staview II were determined by SPSS statistical analysis. $P < 0.05$ was considered significant. Clinical and laboratory data comparison between two *in vitro* lupus subsets was expressed as odds ratio.

Results

In vitro *cytokine production by RA and lupus monocyte subsets, and correlation with clinical and laboratory variables*

To pinpoint a particular cell type responsible for the serum CRP diversification between RA and SLE patients, control and RA monocytes were found to spontaneously secrete only a little IL-6 after a two-day *in vitro* culture (Fig. 1A). Both control and RA monocytes produced comparably high levels of IL-6 upon stimulation with LPS and HSA/anti-HSA immune complexes (IC) at various concentrations (Fig. 1A, 1B, 1C). Serum CRP levels were also high in these RA patients (average CRP = 55.4 mg/L, n = 4) than those two lupus subsets (see below). As expected, and clearly shown in Fig. 1A, control IgG did not significantly change *in vitro* IL-6 secretion by both RA and control monocytes. With all experimental results pooled together, similar results

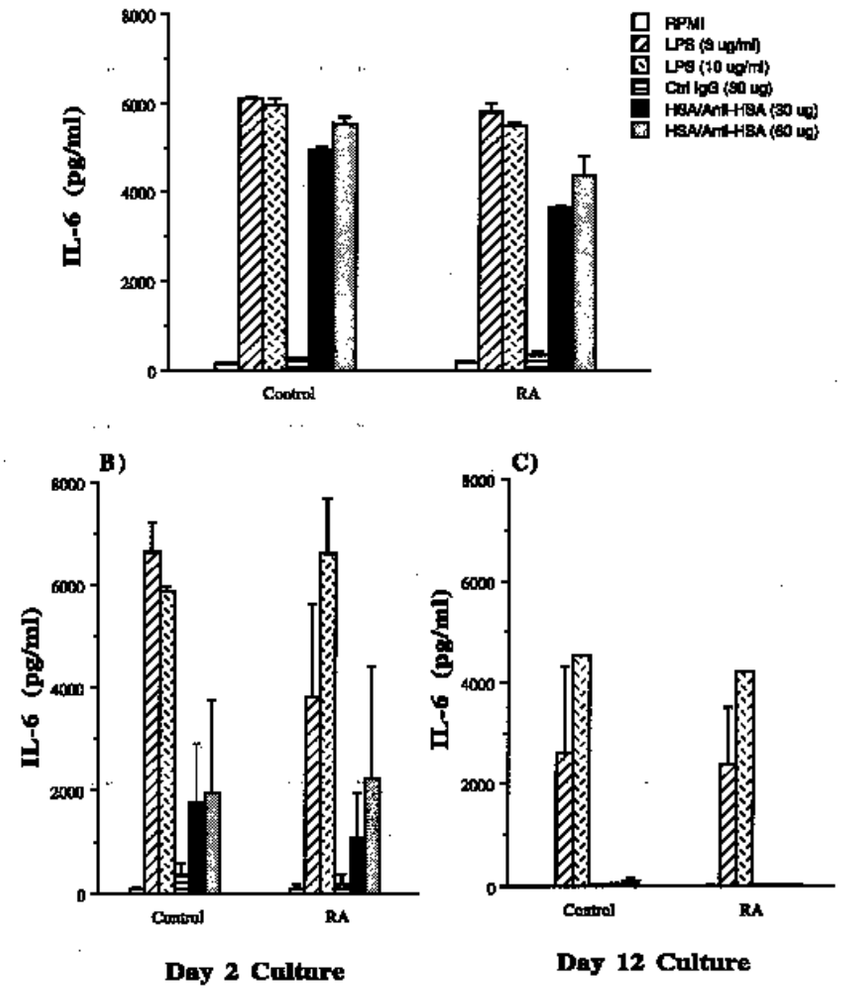

Fig. 1. *In vitro* production of IL-6 from untreated RA monocytes. (A) Representative experimental results on day 2. The inset in the upper right of (A) specifies treatments with various doses of reagents for two days and culture supernatants assayed for IL-6. RPMI refers to cells in medium only; LPS, lipopolysaccharide; Ctrl IgG, control rabbit IgG for anti-HSA antibody; HSA/Anti-HSA, immune complexes. (B) Accumulated results from 4 experiments for day-2 or day-12 cultures. Data are expressed as a mean of means. P = 0.255 is for the comparison between healthy control and RA after LPS 3 μg/ml challenges; the comparison of corresponding LPS 10 μg/ml groups: p = 0.568.

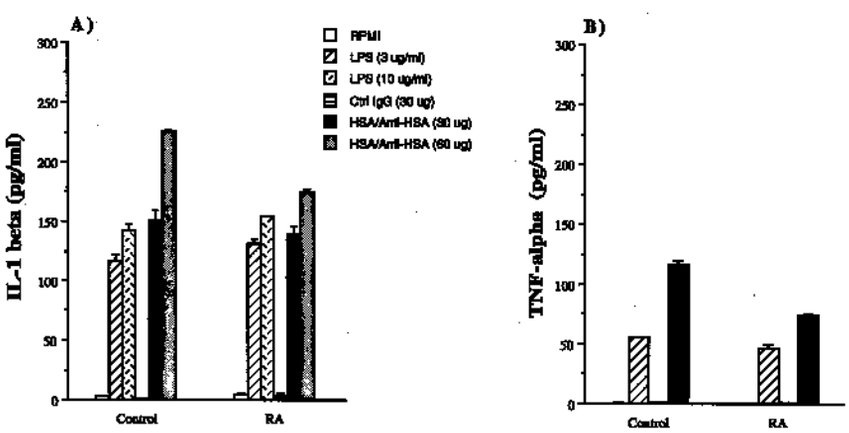

Fig. 2. *In vitro* secretion of IL-1β and TNF-α from a single experiment on day 2 of untreated RA patients. TNF-α generation is much lower in quantity than IL-1β secretion, with the treatments as in Figure 1.

were shown for both day-2 and day-12 cultures with high levels of IL-6 generated at day-two culture and reduced to two-thirds levels on day 12 (Figs. 1B, 1C). The kinetic decline of stimulated IL-6 production was much more prominent in those groups stimulated by IC, almost down to zero at day 12. No significant difference was found between corresponding groups of control and RA monocytes (Fig.1B, 1C). Similar results were also obtained for IL-1β and TNF-α in healthy controls or RA patients (Figs. 2, 3).

Whether a similar trend of secreted cytokines exists for IL-10 was further examined. Though obvious high spontaneous IL-10 secretion was noted from healthy control, RA and SLE monocytes, the responses to HSA/anti-HSA complexes were not different (Fig. 4). However, lupus monocytes produced significantly more IL-10 than those of healthy controls and RA patients, when stimulated by control IgG (Fig. 4). Interestingly, both control and RA monocytes yielded more spontaneous *in vitro* IL-10 in quantity than other cytokines (Figs. 4, 3, 1B). The quantitative order of spontaneous monocyte secretion of cytokines in untreated RA patients was IL-10 >> IL-1β > IL-6 > TNF-α (Figs. 4, 3, 1B). This order, excluding IL-10, was the same as that of the serum cytokine levels in RA patients (4). Hence, it is suggested that monocyte production of cytokines reflect individual cytokine amounts in RA sera. These results implicate indirectly the ability to elicit CRP from RA hepatocytes is not different in terms of the RA disease effect itself or the effect of bacterial infection in RA patients.

However, a different picture from that for lupus monocytes was evident. One subset of lupus monocytes produced much of IL-6 when stimulated by LPS challenge (the high LPS responder), but little IL-6 secretion when stimulated by immune complexes (Fig. 5A, left panel). In contrast, another subset of lupus monocytes reacted only to immune complexes (the high IC responder), but not to LPS (Fig. 5A, right panel). This single example seems to show that the two subsets of lupus monocytes are distinct. Nevertheless,

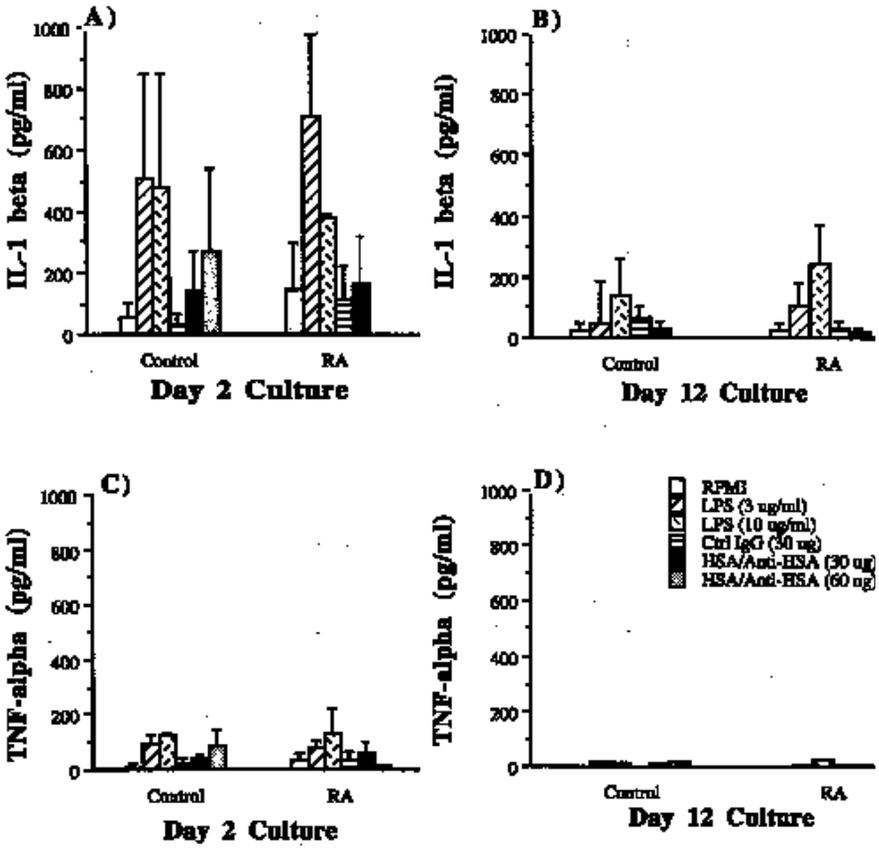

Fig. 3. Cumulative results for IL-1β and TNF-α generation from untreated RA patients. These results are from 4 experiments on day-2 or day-12 cultures. Data are expressed as the means of means. The comparison between healthy control and RA monocytes on day-2 culture after IC 60 μg/ml yields p = 0.665; while the same group comparison for TNF secretion gives p = 0.580.

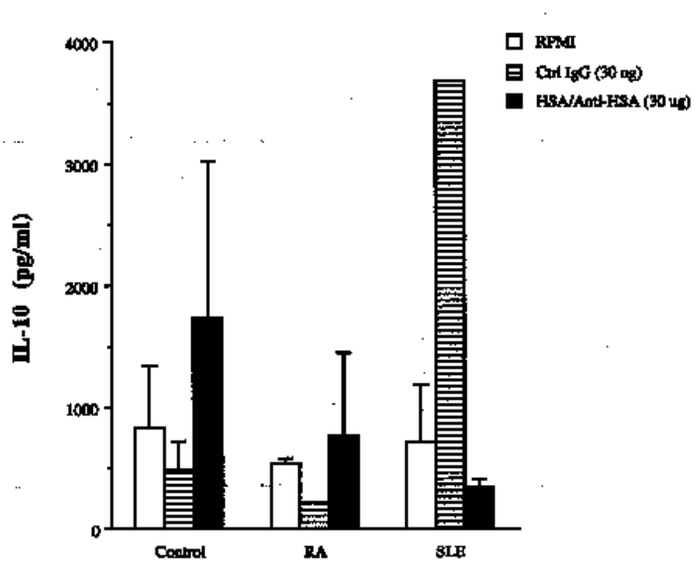

Fig. 4. Cumulated results of IL-10 production by monocytes from the healthy controls (from 4 experiments),untreated RA (2 experiments) and SLE (2 experiments). After culturing with various reagents for two days,supernatants were assayed for IL-10 secretion. Data are expressed as the means of means. After HSA/Anti-HSA stimulation:Healthy control versus RA gives p = 0.622; Healthy control versus SLE with p = 0.512; RA vs. SLE yields p = 0.670. After control IgG (ctrl IgG) challenges:Healthy control versus SLE yields p = 0.009; Healthy control versus RA gives p = 0.632.

analysis of data pooled from all experiments revealed that the high LPS responder subset still reacted to immune complexes, but with much lower IL-6 secretion than that of the high IC responder subset (Fig. 5B). It revealed that a 30 μg IC challenge yielded a difference at p = 0.039 and 60 μg IC gave p = 0.213 (Fig. 5B). These results imply that different amounts of IC may have somewhat varying effects on cytokine generation. The opposite held for the high IC responder subset: LPS-induced IL-6 generation was significantly lower than that of the high LPS responder (p = 0.017, Fig. 5B). The difference between high LPS and high IC responder subsets also existed for the secretion of IL-1β (p = 0.019 for LPS stimulation and $p > 0.05$ on IC challenges), or of TNF-α ($p > 0.05$ for LPS stimulation and $p < 0.05$ for IC challenges) (Figs. 6, 7). Hence, that SLE monocyte subsets respond preferentially to either LPS or immune complexes imply indirectly that CRP production of SLE patients is obviously different from RA patients.

In summary, RA monocytes respond homogeneously to LPS and immune complexes with respect to IL-6, IL-1β, or TNF-α synthesis. However, lupus monocytes responded heterogeneously to LPS and immune complexes. Furthermore, of the lupus subsets, the high LPS responder showed lower mean serum CRP levels (2.55mg/L, odds ratio = 2.25) and a lower SLEDAI (score 4.5, odds ratio = 2.71) than the average serum CRP (5.74 mg/L) and SLEDAI (mean score 12.2) of the high IC responder. No differences were observed for hypocomplementemia, anti-dsDNA abnormality, and positive rate of proteinuria and anti-sm antibody between those two SLE subsets. Whether this diversity of secreted monokines makes any difference in the relationship between the five above mentioned cytokines and CRP in newly diagnosed RA patients from those reported in lupus patients (4) is further examined.

Interrelated association between rheumatoid joint swelling, CRP and serum cytokines

Sera from newly diagnosed, untreated RA patients were measured for various

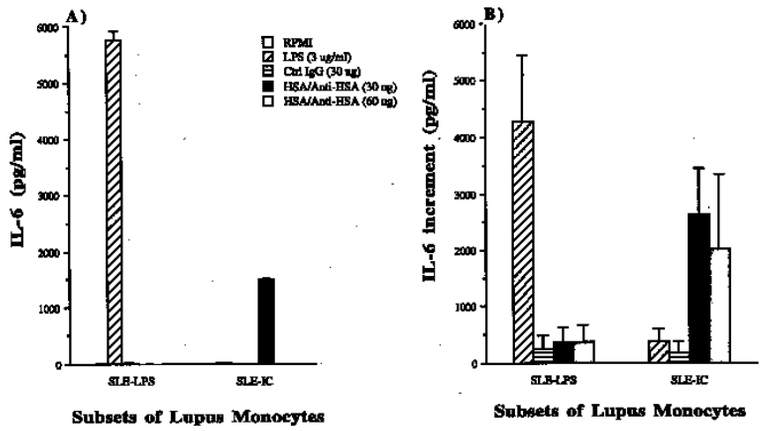

Fig. 5. Various subsets of SLE monocytes. Lupus monocytes were treated as shown in Figure 1 for 2 or 12 days except that no group treated with LPS at 10 mg/ml was present. Culture supernatants were assayed for IL-6. (**A**) Two subsets of lupus monocytes from one representative experiment on day 2 are shown. SLE-LPS designates lupus monocytes that react primarily to LPS; SLE-IC describes lupus monocytes responding preferentially to immune complexes, HSA/Anti-HSA. (**B**) Cumulative results from SLE-LPS (n = 4) and SLE-IC (n = 4) patients as means of means. IL-6 increment indicates the value of a particular treatment group minus the RPMI group.

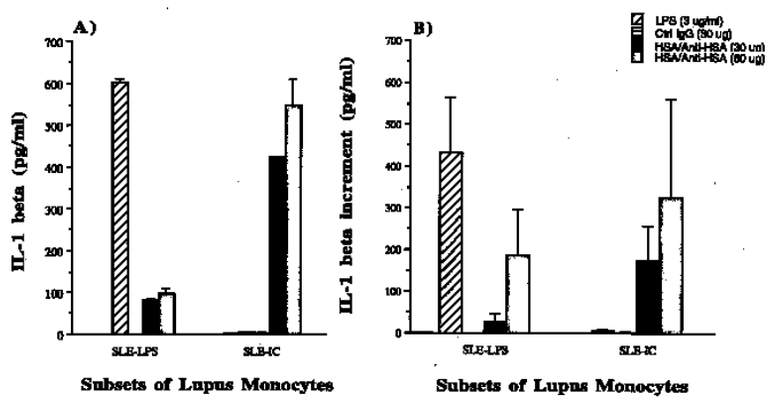

Fig. 6. Secretion *in vitro* of IL-1β from two subsets of lupus monocytes (SLE-LPS with n = 4 and SLE-IC with n = 4). The context is as in Figure 5 and IL-1β increment indicates the value of a particular treatment group minus the RPMI group. P = 0.137 or 0.561 for comparing two subsets treated with HSA/Anti-HSA 30 or 60 μg, respectively.

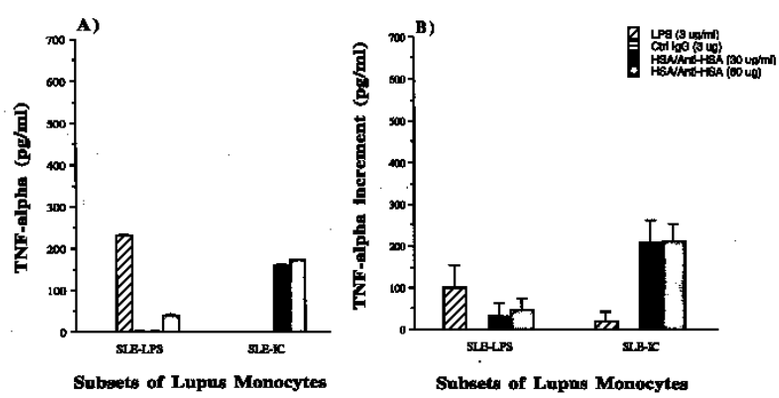

Fig. 7. Generation of TNF-α *in vitro* from two subsets of lupus monocytes (SLE-LPS with n = 4 and SLE-IC with n = 4). The context is as in Figure 5 and TNF-α increment indicates the value of a particular treatment group minus the RPMI group. The comparison for two subsets after LPS stimulation shows p = 0.226. P = 0.028 or 0.031 is for two subsets challenged with HSA/Anti-HSA 30 or 60 μg, respectively.

cytokines to explore the relationship between CRP and CRP-inducing cytokines. CRP was not associated with IL-6, IL-1β, IL-10, or TNF-α (p = 0.386, 0.461, 0.275, or 0.559, respectively). While IL-1ra correlated with CRP at p = 0.088, this borderline association was much stronger than those between CRP and other cytokines (see above). CRP did not significantly relate to the serum rheumatoid factor (RF) (p = 0.479). The association between IL-1ra and other cytokines in RA sera was further examined, as IL-1ra has been shown to be an acute phase reactant *in vivo* in SLE sera (4). Expectedly, serum IL-1ra levels were found to correlate significantly with IL-6, IL-1β, or TNF-α but not IL-10 in RA patients (Fig. 8).

The association between swollen rheumatoid joints and cytokines, CRP, or ESR was then studied, since serum CRP concentrations have been reported to better measure the acute phase response than the erythrocyte sedimentation rate (ESR) in RA patients (21). The swollen joint count was not significantly related to IL-1β, TNF-α, IL-10, IL-1ra, RF, or ESR (p = 0.167, 0.256, 0.093, 0.079, 0.277, or 0.094, respectively). However, the swollen joint count correlated significantly with serum CRP and IL-6 (Fig. 9). ESR did not correlate with IL-6, IL-1β, IL-10, TNF-α, IL-1ra, or RF (all p > 0.2) in RA patients. Hence, serum CRP or IL-6 is a better measure of acute inflammation than ESR in untreated rheumatoid arthritis.

Discussion

Our untreated RA patients do not display the correlation described in earlier reports between IL-6 and serum CRP levels in treated RA patients (11). Furthermore, serum CRP and other cytokines including IL-10 are not correlated. Peripheral blood cytokine concentrations in patients with treated RA are much lower than ours (Fig. 8,9) (11, 17,18). This discrepancy may be explained by the use of corticosteroids (12) or immunosuppressants to repress disease activity of RA in other studies (11, 17, 18). Moreover, most of our RA patients require corticosteroids and/or DMARDs within 2 months (4). Hence, our RA patients differ substantially

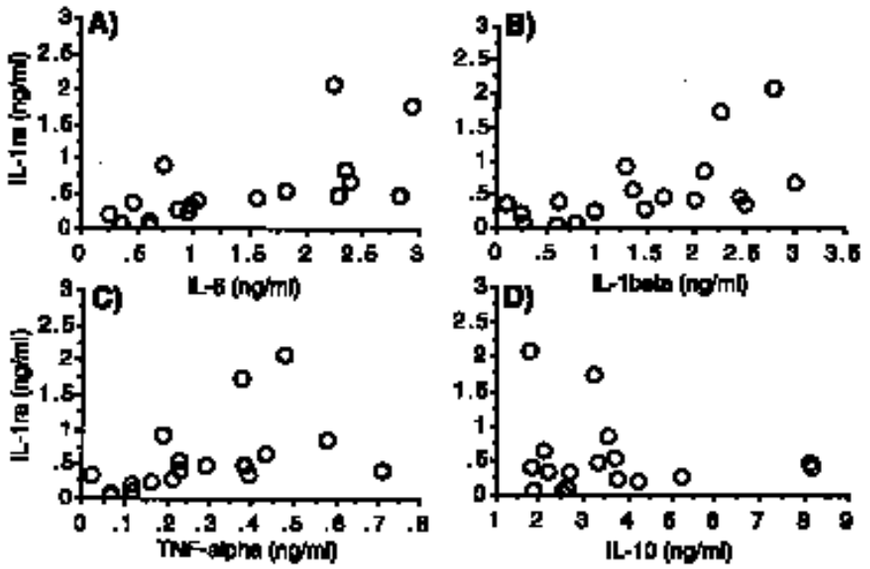

Fig. 8. Relationship of serum IL-1ra levels with serum cytokines in untreated RA patients. (**A**) IL-6 (r = 0.640,p = 0.005), (**B**) IL-1β (r = 0.602,p = 0.008),and (**C**) TNF-α (r = 0.488, p = 0.040) were from 18 serum samples; only 17 serum samples were used for (**D**) IL-10 measurement (r = - 0.140, p = 0.582).

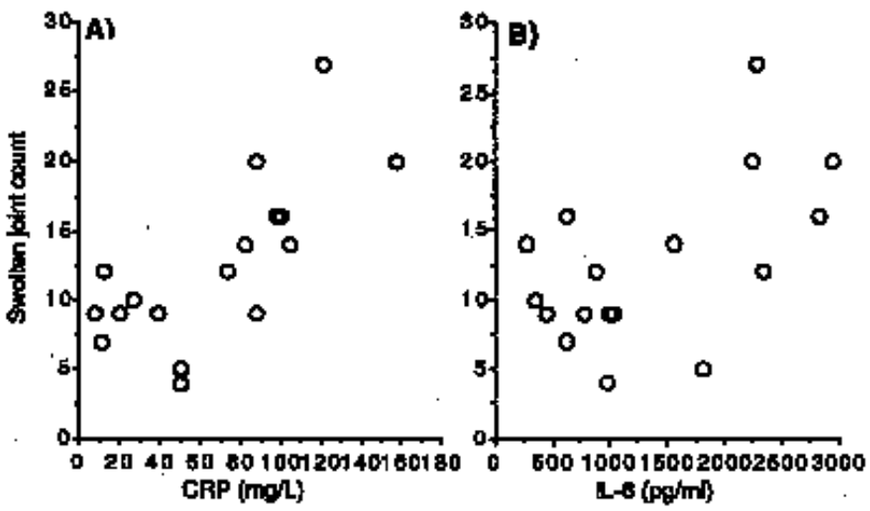

Fig. 9. Correlation of swollen joint counts with serum CRP (r = 0.727, p = 0.001) (**A**) or IL-6 (r = 0.555, p = 0.023) (**B**) concentrations. These are from 17 untreated RA patients.

from those reported RA patients with very mild symptoms, who do not need corticosteroid and/or DMARD therapy for a mean of 9.14 months (18). This could partially explain our high serum cytokine levels, which have yet to be obtained by others in RA patients. Moreover, different assay systems (antibodies from various companies) may yield differing sensitivities. Nevertheless, when compared to those presented by others, our controls revealed very low serum cytokine levels (11, 19). These latter data indicate that our assay system is accurate. Moreover, the high IL-6 concentration secreted from blood monocytes after LPS stimulation in our RA patients (Fig. 1) is similar to that reported earlier (20).

The correlation of swollen joint counts with CRP (Fig. 9A), but not with ESR, in untreated RA patients, supports the previous report that the serum CRP level is a better measure than ESR of the acute phase response in treated RA patients (21). Moreover, the untreated RA patients in this study had at least five times higher serum IL-10 levels (Fig. 8) than did treated RA patients in another report (11), most likely due to corticosteroids or immunomodulating drugs being used in that report (11). Moreover, serum CRP levels related relatively more to serum IL-1ra (p = 0.088) than to other cytokines studied. These findings indicate that two phenomena are shared in two common systemic autoimmune diseases, SLE and RA: firstly, the serum IL-1ra level which correlates with serum CRP is obviously an *in vivo* acute phase reactant in autoimmune diseases. Secondly, serum CRP does not proportionately depend on a single CRP-inducing cytokine to generate itself.

In contrast to immune complex stimulation, day-12 cytokine levels by LPS challenges from monocytes of both groups (control and RA) were quite detectable. These findings show that LPS has a prolonged stimulation effect on RA monocytes to secrete CRP-inducing cytokines than do immune complexes. Interestingly, RA monocytes reacted strongly to immune complexes to produce more IL-6 in quantity than IL-1β and TNF-α (Figs. 1B, 3A, 3C). This results may indicate that RA monocytes require lower doses of immune complexes to secrete IL-6 than that required to secrete IL-1β or TNF-α. The higher quantities of IL-10 than other cytokines spontaneously secreted by the control, RA and lupus monocytes (Fig. 4) probably suggests an autocrine role of IL-10 in human monocytes (22,23). The quantitative order of spontaneous *in vitro* monocyte secretion of cytokines in untreated RA patients was: IL-10 >> IL-1β > IL-6 > TNF-α (Figs. 4, 3, 1B). This quantitative order of spontaneous cytokine secretion from monocytes, excluding IL-10, is the same as that of quantitative serum cytokine orders in RA patients (4). Therefore, it is suggested that monocytes are the most important, but not the exclusive, cell source of serum cytokines.

SLE patients with lower serum CRP levels can be divided into two subsets with respect to *in vitro* monocyte cytokine production (Figs. 5, 6, 7). The

present study suggests that low serum CRP concentrations in SLE patients may be explained by combined (but not individual, see reference 4) low serum cytokine levels that reflect the defective capacity of a subset of lupus monocytes to secrete IL-6, IL-1β, or TNF-α under various conditions. In particular, the high IC responder of lupus patients with higher serum CRP levels (see Results) shows that IC is probably responsible for regular CRP generation (in the absence of infection) by producing IL-6, IL-1β or TNF-α. However, the finding that RA monocytes respond to both LPS (representing a form of bacterial infection) and immune complexes (representing endogenous immune deviation) to synthesize CRP-inducing cytokines implicates this difference from lupus as being responsible for the usually high serum CRP levels in the absence or presence of infection in RA patients (CRP = 55.4 mg/L, much higher than two lupus subsets). Therefore, it is safe to say that monocyte cytokines are responsible for most, but not all of the difference in serum CRP levels between SLE and RA patients.

Moreover, the IC responder subset of lupus patients probably represents the subgroup of lupus having a correlation of elevated blood IL-6 and CRP during exacerbation (24). The percentages of lupus IC or LPS responder subsets among all SLE patients remain unknown and requires study in the future. Nevertheless, there should be a significant portion of lupus patients at different time periods with monocytes that respond only to bacterial infection (LPS) to explain SLE generally having low serum CRP levels in the absence of infection.

In conclusion, the different reaction patterns of untreated RA versus lupus monocytes (Figs. 1A vs. 5A, and so on) suggest that lupus monocyte, rather than RA monocyte, subsets were obviously present. Whether these two SLE subsets have different disease patterns requires further study. In particular, whether these divided subsets exist in the same individual throughout the disease period or are genetically determined remains to be determined. To the best of the author's knowledge, this is the first report in which monocyte reaction patterns explains the heterogeneity of serum CRP levels in systemic lupus.

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References

1. WALSH L, DAVIS P, MCCONKEY B: Relationship between erythrocyte sedimentation rate and serum C-reactive protein in rheumatoid arthritis. *Ann Rheum* Dis 1979; 38: 362-3.
2. HONIG S, GOREVIC P, WEISSMANN G: C-reactive protein in systemic lupus erythematosus. *Arthritis Rheum* 1977; 20:1065-70.
3. ter BORG E J, HORST G, LIMBRUG PC, VAN RIJSWIJK MH, KALLENBERG CGM: C-reactive protein levels during disease exacerbations and infections in systemic lupus erythematosus: A prospective longitudinal study. *J Rheumatol* 1990; 17: 1642-8.
4. LIOULB: Serum and *in vitro* production of IL-1 receptor antagonist correlate with c-reactive protein levels in newly diagnosed, untreated lupus patients. *Clin Exp Rheumatol* 2001; 19: 515-23.
5. AKIRA S, HIRANO T, TAGA T, KISHIMOTO T: Biology of multifunctional cytokines: IL-6 and related molecules (IL-1 and TNF). *FASEB J* 1990; 4: 2860-7.
6. WANIDWORANUN C, STROBER W: Predominant role of tumor necrosis factor-α in human monocyte IL-10 synthesis. *J Immunol* 1993; 151: 6853-61.
7. AREND WP, WELGUS HS, THOMPSON, RC, EISENBERG SP: Biological properties of recombinant human monocyte-derived interleukin-1 receptor antagonist. *J Clin Invest* 1990; 85: 1694-7.
8. BAUMAN H, GAULDIE J: The acute phase response. *Immunol Today* 1994; 2: 74-80.
9. BAUMANN H,GAULDIE J: Regulation of hepatic acute phase plasma protein genes by hepatocyte stimulating factors and other mediators of inflammation. *Mol Biol Med* 1990; 7: 147-59.
10. DEVLIN J, GOUGH A,HUISSOON A *et al.*: The acute phase and function in early rheumatoid arthritis. C-reactive protein levels correlate with functional outcome. *J Rheumatol* 1997; 24: 9-13.
11. LACKI JK,SAMBORSKI W, MACKIEWICZ SH: Interleukin-10 and interleukin-6 in lupus erythematosus and rheumatoid arthritis, correlations with acute phase proteins. *Clin Rheumatol* 1997; 16: 275-8.
12. WAAGE A,SLUPPHAUG G, SHALABY R: Glucocorticoids inhibit the production of IL-6 from monocytes, endothelial cells and fibroblasts. *Eur J Immunol* 1990; 20: 2439-43.
13. KOVALOVSKY D, PAE PEREDA M,SAUER J *et al.*: The Th1 and Th2 cytokines IFN-gamma and IL-4 antagonize the inhibition of monocyte IL-1 receptor antagonist by glucocorticoids: involvement of IL-1. *Eur J Immunol* 1998; 28: 2075-85.
14. DINARELLO CA, THOMPSON RC: Blocking IL-1: Effects of IL-1 receptor antagonist *in vitro* and *in vivo*. *Immunol Today* 1991; 121: 404-9.
15. RIVERO M, SANTIAGO B, GALINDO M, BREHMER MT, PABLOS JL: Cyclooxygenase-2 inhibibition lacks immunomodulatory effects on T cells. *Clin Exp Rheumatol* 2002; 20: 379-85.
16. GABAY C, GENIN B, MENTHA G *et al.*: IL-1 receptor antagonist (IL-1ra) does not inhibit the production of C-reactive protein or serum amyloid A protein by human primary heapotocytes. Differential regulation in normal and tumour cells. *Clin Exp Immunol* 1995; 100: 306-13.
17. LOZA E,TINTURE T, SANCHEZ-IBARROLA A: CD5 and CD23 expression on B cells in peripheral blood and synovial fluid of rheumatoid arthritis patients: Relationship with interleukin-4,soluble CD23 and tumour necrosis factor alpha levels. *Rheumatology* 1999; 38: 325-8.
18. VAZQUEZ-Del MERCADO M,DELGADO-RIZO V, MUNOZ-VALLE JF, OROZCO-ALCALA J, VOLK H-D, ARMENDARIZ-BORUNDA J: Expression of interleukin-1β, tumor necrosis factor α, interleukin-6,-10,and –4, and metalloproteases by freshly isolated mononuclear cells from early never-treated and non-acute treated rheumatoid arthritis patients. *Clin Exp Rheumatol* 1999; 17:575-83.
19. AL-AWADHI A,OLUSI S, AL-ZAID N, PRABHA K: Serum concentrations of interleukin 6, osteocalcin, intact parathyroid hormone, and markers of bone resorption in patients with rheumatoid arthritis. *J Rheumatol* 1999; 26: 1250-6.
20. CHROMARAT P, BANCHEREAU J, MIOSSEC P: Differential effects of interleukin 10 and 4 on the production of interleukin 6 by blood and synovium monocytes in rheumatoid arthritis. *Arthritis Rheum* 1995; 38: 1046-54.
21. WOLFE F: Comparative usefulness of C-reactive protein and erythrocyte sedimentation rate in patients with rheumatoid arthritis. *J Rheumatol* 1997; 24: 1477-85.
22. FOEY AD, FELDMAAN M, BRENNAN FM: Route of monocyte differentiation determines their cytokine production profile: CD4-ligation induces interleukin 10 expression. *Cytokine* 2000; 12: 1496-505.
23. SHIMAUCHI H, OGAWA T, OKUDA K, KUSUMOTO Y, OKAADA H: Autoregulatory effect of interleukin 10 on proinflammatory cytokine production by *Porphyromonas gingivalis* lipopolysaccharide-tolerant human monocytes. *Infection Immunity* 1999: 67: 2153-9.
24. SPRONK PE, Ter BORG EJ, LIMBERUG PC, KALLENBERG CGM: Plasma concentration of IL-6 in systemic lupus erythematosus; an indicator of disease activity. *Clin Exp Immunol* 1992; 90: 106-10.