Neutrophil function in pregnancy and rheumatoid arthritis

I P Crocker, P N Baker, J Fletcher

Abstract

Background—Pregnancy exerts suppressive effects on rheumatoid arthritis (RA). An attenuation in neutrophil function in late pregnancy which may explain this amelioration has previously been reported.

Objective—A longitudinal investigation of neutrophil activity in healthy pregnant women (n=9) and pregnant patients with RA (n=9), compared with age matched non-pregnant patients with RA (n=12) and healthy controls (n=22).

Methods—Neutrophil activation was measured in response to the physiological receptor agonists, n-formyl-methionyl-leucyl-phenylalanine (fMLP) and zymosan activated serum (ZAS). Superoxide anion production (respiratory burst) was determined by lucigenin enhanced chemiluminescence (LUCL); secondary granule lactoferrin release by enzyme linked immunosorbent assay (ELISA); and CD11b, CD18, and CD62L expression by flow cytometric analysis.

Results—Stimulated neutrophil LUCL was significantly reduced in both pregnant women with RA and healthy pregnant women in the second (fMLP 43% and 69%, ZAS 43% and 59%, respectively) and third trimesters (fMLP 24% and 44%, ZAS 32% and 38%, respectively). Responses returned to normal within eight weeks of delivery and unstimulated levels remained unchanged throughout pregnancy. Basal and stimulated CD11b, CD18, and CD62L expression showed no variations throughout gestation for both pregnancy groups. Likewise, stimulated lactoferrin release and plasma lactoferrin remained unchanged. Certain morphological differences in RA neutrophils were highlighted by the flow cytometric analysis. Moreover, resting neutrophils and stimulated cells from patients with RA, including pregnant subjects, showed a marked increase in LUCL, but a reduction in CD11b, CD18, and CD62L. Low dose prednisolone and methylprednisolone had no effect on neutrophil parameters over the period of treatment with non-steroidal anti-inflammatory drugs.

Conclusion—The attenuation to neutrophil respiratory burst in both healthy and RA pregnancies may offer an explanation for the pregnancy induced remission of this inflammatory disorder.

neutrophil derived proteins, such as myeloperoxidase, are detected within joints in a molecular form, suggesting that degranulation has occurred.12

When given this overwhelming evidence for neutrophil involvement in RA, it is not difficult to imagine that any alteration in the adherence, accumulation, or activation of neutrophils in pregnancy may have beneficial effects on the outcome of the disease. Previous studies of functional differences in neutrophils during pregnancy have emphasised a reduction in chemotaxis,13 adherence,14 and microbial killing.15 Moreover, our own studies have shown a depression in neutrophil respiratory burst during pregnancy, which may also explain this ameliorating effect.16

We have previously shown a significant reduction in the receptor mediated respiratory burst activity of neutrophils from pregnant women in the third trimester of pregnancy as compared with non-pregnant controls matched for age.16 In this current study we document the pattern of changes in neutrophil function over the course of pregnancy through longitudinal observations of normal pregnant women and pregnant patients with RA. For these subjects we have compared neutrophil respiratory burst activity, secondary granule lactoferrin release, and the expression cell surface adhesion molecules, CD11b, CD18, and CD62L. These functional markers of neutrophil status were chosen to provide a comprehensive view of ex vivo and, to a limited extent, in vivo neutrophil function. In each case measurements were related to postpartum readings, and also to age and gestationally matched, normal non-pregnant women and RA patient controls.

Materials and methods

SUBJECTS

Women with classical or seropositive RA (American Rheumatism Association criteria) were recruited from the rheumatology clinic of the City Hospital, Nottingham. They comprised two study groups. The first, a group of 12 non-pregnant patients with RA (mean age 29, range 24–33) receiving non-steroidal anti-inflammatory drugs (NSAIDs) but no form of second line treatment; and the second, a group of 9 pregnant patients with RA (mean age 30, range 23–38), two of whom stopped their drug treatment at the start of the pregnancy and seven of whom continued to receive low doses of prednisolone or methylprednisolone (2.5–10 mg/d). Nine women formed a pregnancy group recruited from the antenatal clinic at the City Hospital (mean age 29, range 24–35), while a further 22 healthy, non-pregnant subjects of comparable age (mean age 30, range 24–36) were selected from the hospital staff. Of these, the non-pregnant women were not taking the oral contraceptive pill and all subjects with any pre-existing medical disorders or taking any drugs other than vitamin or iron supplementation were also excluded. Within the two pregnancy groups, three patients with RA and eight healthy women were recruited within the first trimester, and all remaining subjects were recruited in the second trimester. A sample of 30 ml of venous blood was obtained and measurements were recorded every four weeks throughout pregnancy and at approximately six weeks post partum. There were no demographic differences between the subject groups. Informed consent was obtained before inclusion in all cases and the study was approved by the local ethical committee.

PREPARATION OF NEUTROPHILS

Venous blood samples were placed into EDTA-dipotassium salt at a final concentration of 3 mmol/l. Neutrophils were isolated by a rapid, single step technique with Ficoll-Hypaque solution of 1.14 g/ml Polyprep (Nycomed, Birmingham, UK), as described previously.17 Neutrophils were regularly obtained with a purity greater than 97% and a viability greater than 99%. Cells were resuspended in sterile low endotoxin phosphate buffered saline (PBS) pH 7.2 and used immediately.

STIMULATION

The agonists used in the respiratory burst, adhesion molecule expression, and degranulation assays were n-formyl-methionyl-leucyl-phenylalanine (fMLP) (Sigma Chemical Co, Poole, UK) and complement C5a des arg in zymosan activated serum (ZAS). A standard supply of ZAS was produced by the method of Fernandez et al.18 Sterile low endotoxin PBS was used as an unstimulated control.

Table 1

<table>
<thead>
<tr>
<th>Subject group</th>
<th>Total leucocytes (×10⁶/μl)</th>
<th>PMN‡ (×10⁶/μl)</th>
<th>Lymphocytes (×10⁶/μl)</th>
<th>Monocytes (×10⁶/μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy non-pregnant</td>
<td>5.96 (0.62)</td>
<td>3.35 (0.23)</td>
<td>1.82 (0.23)</td>
<td>0.42 (0.05)</td>
</tr>
<tr>
<td>1st trimester pregnant</td>
<td>7.32 (0.68)</td>
<td>4.88 (0.64)</td>
<td>1.84 (0.27)</td>
<td>0.44 (0.04)</td>
</tr>
<tr>
<td>2nd trimester pregnant</td>
<td>7.81 (0.71)</td>
<td>5.61 (0.50)†</td>
<td>1.74 (0.23)†</td>
<td>0.41 (0.05)†</td>
</tr>
<tr>
<td>Postpartum</td>
<td>5.80 (0.54)</td>
<td>2.99 (0.39)</td>
<td>2.18 (0.36)†</td>
<td>0.47 (0.12)†</td>
</tr>
<tr>
<td>RA non-pregnant</td>
<td>8.04 (0.73)</td>
<td>5.02 (0.61)†</td>
<td>1.06 (0.23)†</td>
<td>0.45 (0.07)†</td>
</tr>
<tr>
<td>1st trimester RA</td>
<td>9.80 (0.92)†</td>
<td>5.31 (0.67)</td>
<td>2.89 (0.32)†</td>
<td>0.62 (0.18)†</td>
</tr>
<tr>
<td>2nd trimester RA</td>
<td>12.21 (2.18)†</td>
<td>7.81 (1.16)†</td>
<td>3.06 (1.25)*</td>
<td>0.63 (0.20)†</td>
</tr>
<tr>
<td>Postpartum RA</td>
<td>10.13 (1.03)</td>
<td>6.28 (0.68)†</td>
<td>2.17 (0.30)†</td>
<td>0.38 (0.25)†</td>
</tr>
</tbody>
</table>

Table 1 Pregnancy and rheumatoid arthritis (RA) leucocyte variations. Values are means (SEM)

Statistical significance: *p=0.05 and **p<0.01 compared with postpartum visit; †p<0.05 compared with non-pregnant control group.

‡PMN = polymorphonuclear leucocytes.
reading luminometer. Briefly, 140 µl PBS pH 7.2 containing 1 mM CaCl₂, 0.7 mM MgCl₂, and 0.1% (w/v) low endotoxin bovine serum albumin (PBS/Ca/Mg/BSA), 20 µl of 250 µM lucigenin (bis-N-methylacridinium nitrate), and 20 µl of neutrophil suspension (1 × 10⁷/ml) were added to triplicate wells of a 96 well Immunofluor microtitre plate (Dynatech, Billingshurst, UK). The plates were warmed in the luminometer to 37°C before the addition of either 20 µl fMLP (10 µmol/l) or 20 µl ZAS (50% (v/v)). Chemiluminescence light output was monitored every 60 seconds for 30 minutes and the integral over this period expressed as relative light units (RLUs).

ADHESION MOLECULE EXPRESSION
CD11b, CD18, and CD62L expression were determined by flow cytometry using a direct immunofluorescence technique involving fixation of the cells before antibody staining. This prefixed step prevents the upregulation of β₂ integrin expression as a result of sample handling and has been described in detail elsewhere. ZAS and fMLP were added to anticoagulated (3 mM EDTA) whole blood at final concentrations of 5% (v/v) and 1 µmol/l, respectively. After incubation with end over end rotation at 37°C for 20 minutes, samples were fixed with equal volumes of 1% (w/v) paraformaldehyde (final concentration 0.5%)

Figure 1 Basal neutrophil lucigenin enhanced chemiluminescence and the effect of fMLP stimulation on cell responses from (A) healthy pregnant women (n=9) and non-pregnant controls matched for age (n=22); (B) pregnant RA women (n=9) and non-pregnant patients with RA matched for age (n=12). Integral chemiluminescence light output was recorded as relative light units (RLUs). The non-pregnant results are represent as shaded areas of means (SEM). Longitudinal raw data for pregnant subjects are represented by individual symbols. Stimulated cell responses above resting cell levels are indicated. During the second and third trimester of pregnancy, respiratory burst activity was significantly reduced for both pregnancy groups in response to fMLP and zymosan activated serum (ZAS) stimulation (ZAS results not shown). Neutrophil activity for both pregnancy groups returned to their respective control levels within seven weeks of delivery (see table 2).
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Leucocytosis, and neutrophils the sole source for measurement of secreted lactoferrin. As supernatant was removed and stored at −70°C, they were centrifuged at 400 g with end over end rotation, after which they were incubated for 30 minutes at 37°C or 5% (v/v) ZAS in a final volume of 500 µl.

Table 2 Receptor stimulated neutrophil lucigenin enhanced chemiluminescence. Values are means (SEM) relative light units (RLU)

<table>
<thead>
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<th>Stimulus</th>
<th>Healthy non-pregnant</th>
<th>RA non-pregnant</th>
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<tr>
<td>fMLP†</td>
<td>1272 (226)</td>
<td>1558 (115)</td>
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<td>ZAS‡</td>
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One way analysis of variance analysis; healthy pregnant subjects (fMLP p<0.05, ZAS p<0.01), pregnant subjects with RA (fMLP p<0.01, ZAS p<0.01). Statistical significance; bracketed p values compared with non-pregnant controls, *p<0.05 and **p<0.01 compared with postpartum measurements.

†fMLP = n-formyl-methionyl-leucyl-phenylalanine; ZAS = zymosan activated serum.

Results

Pregnancy and RA haematological variations

Table 1 shows the changes in leucocyte differential blood counts over the course of pregnancy in healthy and RA women as compared with postpartum and non-pregnant controls. During normal pregnancy there was a progressive increase in total leucocyte counts, mainly owing to an increase in the number of circulating neutrophils but also, to a lesser extent, an increase in monocyte numbers in the third trimester. A similar increase in total cell numbers, neutrophils, and monocytes occurred in RA pregnancies, but also with an increase in lymphocytes during the first and second trimesters. All variables returned to control levels after delivery, but baseline numbers of circulating lactoferrin, whole blood counts were carried out to determine the amount of lactoferrin for a standard number of cells.

Table 3 shows the changes in lactoferrin levels in plasma and neutrophil lactoferrin release were expressed as picomoles per 10⁹ neutrophils based on the number of cells in the leucocyte differential counts.

Statistical analysis

Trends over the course of each pregnancy were determined by linear regression analysis. Each subject’s regression coefficient was combined by study group—that is, healthy pregnant or RA pregnant, and then compared by an independent sample Student’s t test. For further statistical analysis, single data points were selected from multiple readings for each subject within the following periods of each trimester: 5–9 weeks, 18–22 weeks, 32–36 weeks, and for 4–8 weeks post partum. For repeated readings, selections were restricted to those nearest to the middle of each time period. The reduced numbers of data points in the first trimesters prevent their inclusion in this statistical analysis, but these measurements are included in the results section. Statistical significance of difference for normally distributed data was determined using either a one way analysis of variance test or Student’s t test (with or without Bonferroni correction). Normal distribution was assessed by the Shapiro-Wilk significance level normality test. The results are presented as means and standard errors of the means (SEM), with the data considered significant at p<0.05.

Table 1 Leucocyte differential blood count

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One way analysis of variance analysis; healthy pregnant subjects (fMLP p<0.05, ZAS p<0.01), pregnant subjects with RA (fMLP p<0.01, ZAS p<0.01). Statistical significance; bracketed p values compared with non-pregnant controls, *p<0.05 and **p<0.01 compared with postpartum measurements.

†fMLP = n-formyl-methionyl-leucyl-phenylalanine; ZAS = zymosan activated serum.
neutrophils were originally higher in the patients with RA than in their equivalent healthy controls.

**EX VIVO SUPEROXIDE ANION PRODUCTION**

In response to the receptor mediated stimuli, fMLP and ZAS, the respiratory burst activity in neutrophils isolated from healthy pregnant women in the second and third trimesters was significantly reduced compared with postpartum measurements and non-pregnant controls (fig 1, table 2). Similarly, responses of patients with RA showed a reduction in respiratory burst over the course of the pregnancy and a return to control levels after delivery (fig 1, table 2). Analysis of the first trimester was excluded because of lower sample numbers. One-way analysis of variance of the remaining trimesters highlighted a progressive reduction in neutrophil responses to fMLP and ZAS, in both healthy pregnant and RA pregnant women. This trend over the course of each pregnancy was further compared by regression analysis, combining the regression coefficients for each pregnant group. The results confirmed no differences between healthy and RA pregnant women in their rates of reduction in neutrophil respiratory burst. There were no significant differences between any postpartum responses and controls, but it was recognised that a significant increase in basal levels of resting cells did exist in all cases between pregnant and non-pregnant patients with RA, and their equivalent healthy controls (statistics not shown).

**ADHESION MOLECULE EXPRESSION**

The use of a rapid leucocyte fixation and preparation procedure allowed for the determination of the unstimulated expression of CD11b, CD18, and CD62L immediately after venesection. The results can be presented as either mean log fluorescence units or as the percentage of neutrophils positive for the fluorescence antibody. Neutrophil CD11b and CD18 expression were increased by activation with chemotactic agents (fig 2). Conversely, CD62L is down-regulated during activation and responded to stimulation by a significant decrease in percentage positive cells (fig 3) and mean fluorescence units (fig 2). For CD11b and CD18 expression the mean fluorescence measurements are given for positively stained neutrophils. This population routinely contained 98% of all neutrophils counted. As shown in fig 2 there were no differences in the unstimulated or stimulated expression of CD11b, CD18, or CD62L between the normal non-pregnant measurements and the normal pregnant and postpartum values. Similar results were recorded for RA pregnant and non-pregnant groups, showing no differences in both stimulated and unstimulated expression for all three adhesion molecules studied. In addition, neutrophils from non-pregnant RA women expressed less CD11b, CD18, and CD62L overall than their equivalent controls. However, when presented as the percentage of cells expressing CD62L there was no significant difference between healthy controls and patients with RA, whether pregnant or not (fig 3). The fixation method used had no effect on adhesion molecule expression over the experimental time period (data not shown).

**LACTOFERRIN RELEASE**

Direct measurements of plasma lactoferrin concentrations showed a significant difference between the healthy and RA non-pregnant groups (2.55 (0.41) nmol/l and 3.95 (0.58) nmol/l (p<0.01), respectively; fig 4). However,
when values were normalised to account for the number of circulating neutrophils, equivalent readings were achieved (fig 5). For measurements of normal pregnant versus RA pregnant whole blood cells, the values of lactoferrin released in response to fMLP and ZAS showed variations between trimesters and also between subject groups (fig 4). Again these differences were abolished when the number of lactoferrin producing neutrophils present were accounted for and the measurements were expressed as lactoferrin released (pmol/10^6 cells; fig 5).

**LIGHT SCATTERING PROPERTIES**

The flow cytometer allows the visual representation of cell populations according to their light scattering properties. For neutrophils, a forward and side scatter value will give a crude but measurable indication of both cell size and cell complexity. Therefore, morphological changes during neutrophil maturation and activation can be evaluated, at least to a limited degree, by the assessment of these light scattering characteristics. For example, an increase in forward scatter and a concomitant decrease in

![Figure 3](image1.png)

**Figure 3** Expression of CD62L on the surface of neutrophils from women of the four study groups. The results show changes in the percentage of positive cells present before stimulation (phosphate buffered saline) and after stimulation with fMLP or zymosan activated serum. Data are expressed as mean values (SEM).

![Figure 4](image2.png)

**Figure 4** Plasma lactoferrin and unstimulated (phosphate buffered saline) and stimulated (fMLP, zymosan activated serum) whole blood lactoferrin release from non-pregnant and pregnant healthy women, and from non-pregnant and pregnant patients with RA matched for age. Data are shown as mean (SEM) concentrations (nmol/l). Statistical significance of difference was determined between the RA groups and their equivalent healthy, non-pregnant and pregnant control groups. The degree of significance is indicated by *p<0.05 and **p<0.01 (t test).
side scatter readings are always observed when cells undergo degranulation, either through “priming” or activation by an appropriate stimulus. In the case of our four study groups the mean forward scatter intensity, representative of cell size and dimensions, showed no differences between cells from both RA and/or pregnant states. However as fig 6 indicates, side scatter analysis of these same cells did show a decrease in cell complexity for the RA group compared with the non-pregnant controls. Although paraformaldehyde fixation can affect neutrophil light scattering properties all cells were fixed and treated in the same way. Therefore, this observation may be highlighting a morphological difference in the neutrophils of patients with RA, one which persists throughout all stages of pregnancy.

**Discussion**
We have previously shown alterations to neutrophil function during healthy pregnancy. The objective of this study was to confirm whether these changes occur in pregnant patients with RA, and therefore provide an explanation for the ameliorating affects of pregnancy on RA. To achieve this objective we investigated neutrophil respiratory burst activity, adhesion molecule expression, and lactoferrin release in pregnant
and non-pregnant patients with RA and healthy subjects.

Neutrophil respiratory burst activity was measured in response to physiological stimuli using lucigenin, a chemiluminescent probe selective for extracellular superoxide anions.\textsuperscript{22} Our findings suggest that although basal neutrophil responses remain unaltered, stimulated respiratory burst activity is diminished throughout the course of pregnancy. This decline in activity is characterised by a gradually depression from the second trimester followed by a return to preconception and control levels within eight weeks of delivery. Although consistent with earlier studies of superoxide production, these results are in conflict with studies employing less direct measurements of respiratory burst. For instance, studies using luminol chemiluminescence have shown a trend towards increased activity during pregnancy,\textsuperscript{23} whereas those employing more selective measurements of superoxide anions, such as lucigenin, have shown a consistent reduction.\textsuperscript{24} A previous in vitro observation by Buyon et al has highlighted the inhibitory effect of oestriadiol on neutrophil superoxide generation.\textsuperscript{25} In light of these findings, and in support of this study, it must be emphasised that the timing of the diminished responses to FMLP and ZAS is consistent with reported clinical observations of reduced symptomatic pain relief in RA.\textsuperscript{7} It must also be recognised that these results are in agreement with an inhibitory effect of pregnancy sera on the phagocytosis of viable bacteria,\textsuperscript{26} and also with the increased incidence of specific intracellular infections during the latter part of gestation.\textsuperscript{4}

In addition to the ability of neutrophils to become activated in response to inflammatory stimuli, it is widely recognised that bloodstream neutrophils can also be primed by sub-optimal concentrations of stimuli, or by exposure to relevant inflammatory cytokines. More simply, any form of priming in vivo, or in vitro, results in molecular modifications to neutrophils and an accompanying enhancement in their functional responsiveness. In RA, an increase in the levels of cytokines has been identified through a rise in the circulating concentrations of tumour necrosis factor \( \alpha \) and interleukin 6.\textsuperscript{27} Although these cytokines are enhancers of stimulated neutrophil lucigenin chemiluminescence in vitro,\textsuperscript{28} 29 we have previously reported a decline in tumour necrosis factor \( \alpha \) and interleukin priming of neutrophils from the third trimester of pregnancy.\textsuperscript{16} The new data from this study indicate that the resting cell responses for healthy pregnant and non-pregnant subjects remain consistent, but for patients with RA these same cell responses are considerably raised. Although this might represent a partial “priming” of bloodstream neutrophils in the disease state, the observed alterations in neutrophils during pregnancy are nevertheless reaffirmed. Pregnant patients with RA showed the same reduction in respiratory burst and subsequent return to non-pregnant control levels as those of equivalent healthy pregnancy cells.

In RA, an extensive granulocytic infiltration of the pannus and periarticular tissues, coupled with the prevalence of proinflammatory cytokines and immune complexes in the synovial fluid (SF), provides a microenvironment compatible with neutrophil activation. In support of this inflammatory model, preparations of neutrophils isolated from affected joints show convincing evidence of in vivo activation.\textsuperscript{30} In addition to RA, it has also been suggested that circulating neutrophils in pregnancy are activated and as a consequence show reduced phagocytosis ex vivo.\textsuperscript{31} Experimentally, neutrophil priming and activation can be best identified by a transformation in cellular adhesion molecule expression—most notably by a rise in the \( \beta_2 \) integrin, CR3 (CD18/CD11b) expression, and a fall in L selectin, CD62L. The \( \beta_2 \) integrins are up-regulated on the cell surface through degranulation,\textsuperscript{32} whereas CD62L is constitutively expressed and is shed upon cell activation/priming by proteolytic cleavage.\textsuperscript{33} Our data show that although the expression of CD18, CD11b, and CD62L on unstimulated neutrophils is unaltered in pregnancy, differing observations are evident for pregnant and non-pregnant patients with RA. No marked differences in the patterns of responsiveness are apparent in the unstimulated responses of both pregnant and non-pregnant RA groups, but basal levels of expression in RA of all adhesion molecules examined were significantly lower. Although a surprising observation, which conflicts with other studies,\textsuperscript{34} similar results have previously been reported.\textsuperscript{35} 36

A possible clue to this expression of adhesion molecules is given by the light scattering properties of RA cells within the flow cytometer. The measured decrease in side scatter, and absence of an accompanying forward scatter transformation, is a new finding for neutrophils. Although unusual and open to interpretation, this observation strongly implies morphologically different neutrophils in pregnant and non-pregnant patients with RA.

Lactoferrin, a widely distributed protein, stored predominantly in the specific granules of neutrophils, has been shown to be released during normal neutrophil activation in vitro.\textsuperscript{37} Together with elastase—a proteolytic enzyme stored in the azurophil granules—high concentrations of lactoferrin are evident in affected joints,\textsuperscript{40} 41 and raised circulating levels have been shown in RA.\textsuperscript{42} Strong correlations with elastase-proteinase inhibitor complex and C reactive protein measurements of disease activity have advanced lactoferrin as a possible marker for neutrophil dependent inflammation in RA. This study has shown increased levels of plasma lactoferrin in RA and the latter stages of pregnancy, and has also highlighted an increase in lactoferrin upon whole blood receptor stimulated release.

Although on first inspection these results may indicate prior activation in vivo and a primed responsiveness of cells in vitro, the authors believe a cautionary note must be added to this interpretation. In the past, both plasma lactoferrin in patients with cyclic neutropenia,\textsuperscript{43} and plasma and SF lactoferrin.
and elastase in patients with RA, have all been correlated significantly with absolute neutrophil counts. In fact, when our results are corrected for circulating lactoferrin producing neutrophils, no discernible differences in plasma measurements or cellular responses could be established between the study groups. It may be concluded that for this study the raised plasma levels in pregnancy and RA are a direct reflection of neutrophil numbers and turnover and not, as previously assumed, a result of either cellular degranulation or activation in vivo.

Although the functional status of SF derived neutrophils is difficult to dispute, it is currently contentious whether full or partial activation occurs in circulating neutrophils of patients with RA. Previous studies claim that certain drugs used in the treatment of RA may moderate peripheral blood neutrophil numbers and responsiveness. Certainly, glucocorticoid treatments will raise neutrophil numbers, but their ability to alter cellular function remains undetermined. In vitro observations suggest that neutrophils are largely resistant to therapeutic levels of methylprednisolone and other antirheumatic drugs. However, similar in vivo studies suggest a decrease in neutrophil ingress and an associated decrease of CD11b and CD18. Obviously, the recruitment of subjects with active RA who are not taking drugs is difficult, and this becomes even more confounded when restricted to women of childbearing age. In this study the combination of treatments and groups allows some conclusions to be drawn. The postpartum readings of the pregnant RA group provide a direct comparison between two sets of non-pregnant patients with RA, one group taking NSAIDs and the other methylprednisolone/ prednisolone. As differences between these groups were not identified, it can be said that prednisolone has no affect on neutrophils above that offered by NSAIDs. Given that the action of glucocorticoids and NSAIDs on neutrophil function remains debatable, a full interpretation of their effects is difficult. These agents may alter adhesion molecule expression, but it seems unlikely that the neutrophil respiratory burst will be affected by these treatments. Similarly, glucocorticoids may explain the raised neutrophil levels in the patients with RA studied, but NSAID treated subjects, as well as those taking prednisolone, also showed raised neutrophil numbers. As NSAID treatment is unlikely to affect neutrophil numbers, a more plausible explanation for this and other observations is that changes in neutrophil behaviour are a consequence of disease activity rather than drug treatment.

In this study the pattern of adhesion molecule expression on neutrophils from patients with RA suggests morphologically different cells with functionally normal or even primed responses. Moreover, similarities in cellular activities and adhesion molecule markers indicate that any proposed in vivo activation or priming of neutrophils does not occur in normal pregnancy. The observed reduction of neutrophil superoxide anion release in pregnant patients with RA occurs regardless of their drug treatment and is a particularly interesting observation. Superoxide anions act as precursors for a number of reactive oxygen species that are thought to be important in degrading protective proteins and directly contributing to tissue destruction. Undoubtedly, any pregnancy associated depression of neutrophil function might conceivably reduce the degree of SF inflammation and thereby contribute to the improvement of RA. Currently, efforts are underway to establish a rationale for the observed changes in the respiratory burst of neutrophils in pregnancy.
elicited by the chemotactrant n-formylmethionyl-


31 Emery P, Lopez AF, Burns GF, Vadas MA. Synovial fluid neutrophils of patients with rheumatoid arthritis have membrane antigen changes that reflect activation. Ann Rheum Dis 1986;47:34–9.


