Increased expression of Fcγ receptors on neutrophils and monocytes may reflect ongoing bacterial infection

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The FcγR receptors for IgG, FcγRI, FcγRII and FcγRIII were measured on neutrophils and monocytes from 36 patients suspected of systemic infection. These results were compared with 30 blood donor controls to assess the level of expression as an early indicator of bacterial infection. FcγRI expression on neutrophils was found to be significantly increased from patients with systemic or localised infections, when compared to the non-infected patient group, i.e., patients with no cultural evidence of bacterial infections, \( p = 0.02, p = 0.04 \) or normal controls \( p < 0.0001, p = 0.0005 \). FcγRI expression on monocytes was also significantly increased in both of the infected groups compared to normal controls \( p < 0.0001, p = 0.001 \); however, no significant difference could be seen when compared with the non-infected patients. FcγRIII was found to be significantly increased on a subset of monocytes in patients with systemic or localised infections compared to the non-infected group \( p = 0.009, p = 0.006 \) and compared to the normal controls \( p = 0.009, p = 0.003 \). Infections caused by gram-negative bacilli induced a higher FcγR response than infection with either streptococci or staphylococci. These data suggest that the measurement of FcγRI on neutrophils and FcγRIII on monocytes may be a useful rapid indicator of bacterial infection.

Introduction

Despite appropriate antimicrobial chemotherapy, one of the most frequent and serious problems facing clinicians is the management of patients with severe infection [1, 2]. Prompt diagnosis is essential to improve prognosis. The development of methods for the early detection of infection based on the measurement of host immune response, rather than sometimes lengthy culture techniques, would be advantageous.

Human phagocytes express three distinct classes of receptors for the Fc domain of IgG designated FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16) [3, 4]. In the body, these receptors form a two-way bridge between the humoral response and the cellular branch of the immune system [5]. When Fcγ receptors (FcγR) interact with immunoglobulin, various important biological responses are triggered, such as phagocytosis, endocytosis, antibody-dependent cellular cytotoxicity, release of inflammatory mediators and enhancement of antigen presentation [6]. Therefore, it is expected that as these receptors and their regulation have a priority in infection, the cellular immune response to bacterial challenge would involve increased expression of FcγR on the phagocytic cells, at an early stage of infection.

Early studies have already indicated that FcγRI expression is increased in acute bacterial infection [7]. Other studies have shown that IFN-γ can bring about a dramatic increase in FcγRI expression on both monocytes [8] and neutrophils [9]. More recent work has also shown that FcγRI expression is increased on neutrophils and augmented on monocytes in patients with localised β-haemolytic streptococcal (potent IFN-γ inducers) pharyngitis [10]. This study was designed to investigate whether FcγR expression is also increased in systemic infection and, if so, whether its measurement could be used as an early indicator of infection.

Materials and methods

Patient population

Whole blood EDTA samples were collected from 36 patients with suspected systemic infection, at the time of blood culture analysis. Thirty EDTA samples were also collected from blood donors as healthy controls. Of the 36 patients tested, 16 had systemic infection, i.e., clinically significant bacteria were isolated from the blood culture. These isolates consisted of the
following: Staphylococcus aureus (n = 5, four of which were methicillin- and gentamicin-resistant strains); Streptococcus pneumoniae (n = 2), Enterococcus sp. (n = 1), Escherichia coli (n = 5), Enterobacter cloacae (n = 1), Klebsiella oxytoca (n = 1) and Pseudomonas putida (n = 1). Six patients were blood-culture negative but had severe localised bacterial infections at other sites. Of these patients, four had infections in the lower respiratory tract caused by gram-negative pathogens such as K. pneumoniae, P. aeruginosa, E. coli and Haemophilus influenzae. Another of these patients had peritonitis due to E. coli whilst the remaining patient had Clostridium difficile-associated diarrhoea. The remaining 14 patients tested had no cultural evidence of bacterial infection. Viral studies were not performed on the patients tested.

**Fluorescent staining**

FcγR expression was detected by a direct staining immunofluorescence technique with fluorescein isothiocyanate (FITC)-labelled monoclonal antibodies (MAbs) against FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16) (Medarex, Princeton, NJ, USA). Phycoerythrin (PE)-labelled CD14 (BD) was used as a monocytic marker. A non-sense antibody, γγ2 (BD) was also used as a control for non-specific binding. The receptors were stained by a whole blood lysis technique on a single EDTA specimen which was divided into four 100-μl volumes. Five μl of the three FcγR antibodies were added to three of the tubes, respectively. Non-sense antibody (5 μl) was added to the fourth tube. A further 5 μl of the CD14 antibody was added to each tube. After incubation for 10 min at room temperature, 2 ml of red cell lysis solution (BD) were added to each tube and tubes were incubated for a further 10 min. Tubes were centrifuged at 5000 rpm for 5 min after which the supernate was discarded. Samples were then washed in FACS flow fluid (BD), and fixed in paraformaldehyde 1% in PBS.

**Flow cytometric analysis**

Flow cytometric measurement of the stained samples was performed with a single laser (Argon ion 488 nm) FACSCAN flow cytometer (BD). CaliBRITE beads (BD) were used daily to set the PMT voltages and the fluorescent compensation and also as a check on instrument sensitivity. Patient samples were acquired at a rate of c. 200 cells/s, such that 10,000 events were counted on each sample. LYSIS software was then used to estimate MAb binding on the neutrophils and the monocytes. Neutrophils were distinguished and electronically gated on the basis of their size and granularity, by means of a dot plot of forward scatter (FSC) versus side scatter (SSC). Gating on the PE-labelled monocytes was performed with a dot plot of the fluorescent intensity of FITC (FL1) versus the fluorescent intensity of PE (FL2). The mean fluorescence intensity (MFI) of each of the cell populations was then calculated from a profile histogram of the FL1 for each sample. For FcγRIII, which is expressed only on a subpopulation of monocytes, the percentage of monocytes expressing this receptor was also calculated from the FL1 histogram. A marker was set on the subpopulation of monocytes with increased FcγRIII expression, against a background control, and the percentage of cells within the marked region was then calculated with the 'Histats' in the LYSIS programme. The percentage of monocytes with increased expression of FcγRIII was calculated for each of the patient samples, but on a smaller number of controls (n = 15) than originally tested.

**Statistical analysis**

Mean MFI values for the three receptors, on both neutrophils and monocytes, were calculated for each of the patient groups and the controls. MFI values for the patient groups and the controls were also compared by analysis of variance (ANOVA) and a modified t test for unpaired samples, based on a pooled estimate of variance. A p value <0.05 was taken to be significant. Similar calculations, including patient group mean values and t test comparisons, were performed on the percentage of monocytes with increased expression of FcγRIII.

**Results**

Examination of MFI values indicated that whilst FcγR expression was increased in patients with either systemic or localised infection, there was little difference in expression between the non-infected patients and the controls (Table 1). There was no significant difference in the level of expression of FcγRI, FcγRII or FcγRIII in patients with either systemic or localised infection. However, for FcγRI expression, there was a significant difference between patients with either systemic or localised infection, compared to the controls, for both neutrophils (p < 0.0001, p = 0.0005) (Fig. 1A) and monocytes (p < 0.0001, p = 0.001) (Fig. 1B). FcγRII expression was also significantly increased in the two infected patient groups compared to patients without evidence of infection, for neutrophils (p = 0.02, p = 0.04) (Fig. 1A). The monocytic expression of FcγRIII was also significantly increased in patients with either systemic or localised infection compared to controls (p < 0.0001, p < 0.0001). This increased expression of FcγRIII in infection was more apparent when comparisons were made by calculations involving the percentage of monocytes expressing increased levels of this receptor. In this case the percentage of monocytes showing a higher level of FcγRIII expression was significantly increased in both infected groups compared not only to the controls (p = 0.009, p = 0.003), but also to patients without evidence of infection (p = 0.009, p = 0.006) (Fig. 1C).
Table 1. Mean fluorescence intensity (MFI) values for Fcγ receptor expression on neutrophils and monocytes in patient and control groups.

<table>
<thead>
<tr>
<th>Patient groups</th>
<th>Neutrophils</th>
<th>Monocytes</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>FcγRI</td>
<td>FcγRII</td>
</tr>
<tr>
<td>Control</td>
<td>26 (21–31)</td>
<td>120 (105–135)</td>
</tr>
<tr>
<td>Systemic infection</td>
<td>62 (34–90)</td>
<td>147 (103–191)</td>
</tr>
<tr>
<td>Localised infection</td>
<td>76 (43–109)</td>
<td>161 (130–192)</td>
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*Controls: blood donors; non-infected patients: patients without cultural evidence of bacterial infection; systemic infection: patients with systemic infection; localised infection: patients with negative blood culture but significant infection at localised sites.

Analysis of the MFI values obtained in patients with systemic infection also indicated that some bacterial isolates induced a higher FcγR response than others. For FcγRI, on both neutrophils and monocytes, gram-negative bacilli appeared to elicit a higher receptor response than either streptococci or staphylococci (Fig. 2A). Similarly, for FcγRIII, the percentage of monocytes with increased expression of this receptor, showed a notable increase in infections caused by gram-negative bacilli (Fig. 2B). In turn, for both FcγRI and FcγRIII, *Streptococcus* spp. such as *S. pneumoniae*, induced a higher FcγR response than most of the staphylococcal isolates. The gram-negative organisms involved in localised infections also induced a high FcγR response, but at a lower level than the gram-negative organisms causing systemic infection.

**Discussion**

It is now recognised that the high mortality rate associated with systemic infection arises not only from the presence and establishment of organisms in the bloodstream, but also from the body's own immune response to the infection, a response that can trigger a potentially fatal cascade of inflammatory mediators [1, 11, 12]. Terms such as sepsis, sepsis syndrome and septic shock are no longer used to denote separate infectious disorders. These terms are now used to represent increasingly severe stages of the same infectious process, the progress of which relates to the severity of the systemic response rather than to the infection itself [13].

The development of suitable antimicrobial therapy has brought about a great reduction in the mortalities associated with systemic infection. However, administration of effective antimicrobial agents can have many contraindications for the patient. In gram-negative infection, for example, septic shock can be triggered as a primary response to the release of lipopolysaccharide (LPS) from the cell wall, following antimicrobial therapy [14, 15].

Recent research in the management of systemic infection has concentrated on the development of therapy, such as anti-cytokine strategies, to modulate the immune response to infection and to dampen the inflammatory cascade [16]. Indications from preliminary immunotherapy trials suggest that timing is critical, with improved responses resulting from early therapeutic intervention [13]. The development of methods for the early detection of infection, based on the responses of the immune system itself, would clearly be of great advantage [17]. Many studies have already considered the measurement of pro-inflammatory cytokines such as IL-6 [17], TNFα, IL-1β [18] and IFN-γ [10], as indicators of infection. However, determination of these cytokines may not reflect their true level of cellular activity, as serum concentrations may be below detection [10]. Furthermore, the methods of measurement are difficult and the cytokines are of a very labile nature [10]. Therefore, determination of the cellular response to cytokine release may prove a more successful indicator of the immune response.

The results of this study indicate that measurement of the Fcγ receptor response may be used as an early indicator of infection. The expression of FcγRI and the percentage of monocytes demonstrating upregulation of FcγRIII, were significantly increased in patients with either systemic or localised infection, compared to patients without evidence of bacterial infection and controls. These results are in agreement with previous studies investigating Fcγ receptor response. In 1993, Guyre *et al.* also found that FcγRI expression was increased on neutrophils and augmented on monocytes in patients with localised group A β-haemolytic streptococcal pharyngeal infection [10].

In a recent study involving HIV-infected patients, the expression of FcγRI and the percentage of monocytes expressing FcγRIII were also found to be significantly increased, compared to controls [19]. In a further study involving HIV-infected patients, the level of expression of FcγRI and the percentage of monocytes expressing FcγRIII were not only found to be significantly increased, but were also found to have an association with serious or opportunistic infections in these patients (unpublished observations).
Fig. 1. Fcγ receptor expression in patient and control groups: a, mean fluorescence intensity (MFI) values for FcγRI on neutrophils; b, MFI values for FcγRI on monocytes; c percentage of monocytes with increased FcγRIII expression. Patient groups consisted of non-infected patients, patients with systemic infection and patients with localised bacterial infection; p values are quoted where there is a significant difference (p < 0.05) between groups.
patients with either localised or systemic infection. However, increased FcyR expression may not occur in all bacterial infections. In a study involving streptococcal infection, Guyre et al. [10] suggested that FcyRI was selectively increased in some bacterial infections. They found that FcyRI expression was upregulated in a small number of patients with pyelonephritis compared to patients with gram-negative urinary tract infections [10]. This might suggest that in localised infection the level of FcyR expression will depend on the level of systemic involvement. In the present study, the localised infections examined were of a severe nature, involving gram-negative lower respiratory tract pathogens, and, as a result, may have been sufficient to activate the entire circulating pool of phagocytes. Therefore, these results may indicate that, whilst the measurement of FcyR expression may not distinguish the site of infection, be it bloodborne or localised, measurement could help to indicate the type of infection, in terms of the extent of systemic involvement.

Furthermore, in preliminary studies performed on a patient with gram-negative systemic infection, the FcyR response appeared to parallel the course of infection (unpublished data). In the initial period of
infection the FcγR expression was significantly increased. With resolution of infection, after administration of suitable antimicrobial therapy, the levels returned to control values, suggesting that the measurement of FcγR responses could also be of prognostic value.

The results of the present study also indicate that certain types of bacteria can elicit a higher Fcγ receptor response than others. In patients with septicemia, infections caused by gram-negative bacilli such as *K. oxytoca, Ent. cloacae* and *E. coli* induced a higher increase in Fcγ receptor expression than either streptococci or staphylococci. In turn, systemic infections caused by streptococci such as *Str. pneumoniae*, induced a higher response than infections involving *S. aureus*. Similarly, in localised infection, gram-negative bacteria also induced high levels of FcγR expression, but to a lesser degree than gram-negative isolates in systemic infection. These results suggest that the measurement of the level of FcγR expression could be used to indicate the type of pathogen involved.

Increased FcγR expression is thought to result from the release of IFN-γ from activated T cells after antigenic challenge [8, 9]. Therefore, during infection, the level of FcγR expression will depend on the ability of the organism to induce IFN-γ release. In the case of gram-negative bacilli, bacterial LPS interacts with LPS-binding protein, through the CD14 glycoprotein, to bring about release of pro-inflammatory cytokines with subsequent T-cell activation [13]. However, recent studies have indicated that LPS can act directly on natural killer cells, to produce an additional source of IFN-γ [20]. Taken together this could explain the high level of FcγR expression observed in gram-negative infection in this study. In the case of infection caused by gram-positive organisms such as streptococci, increased levels of IFN-γ could result from the action of superantigens, e.g., exotoxins and certain M proteins. By circumventing the normal rules of antigen presentation and T-cell recognition, these antigens can stimulate large numbers of T lymphocytes and thus amplify the release of cytokines [21]. Studies of bacteria-induced FcγR expression could further our understanding of the inflammatory response in infection.

The results of this preliminary study suggest that the measurement of FcγR expression could be used as an early indicator of infection. Quantification of the FcγR response might also help to indicate the type or severity of infection and even the organism involved. Furthermore, as the method of determination of FcγR is both rapid and simple to perform, this test could be used easily in conjunction with standard culture technique and could serve as a useful tool in the treatment of infection.

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**References**