Invasive fungal infections are associated with severe depletion of circulating RANTES

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INTRODUCTION

Risk factors for invasive fungal infections (IFIs) and their progression include protracted neutropenia and phagocyte functional defects (Schaffner et al., 1982; Gerson et al., 1984). Polymorphonuclear cells (PMNs) are rapidly destructive to fungal hyphae in particular through toxic oxygen radicals (Rex et al., 1990). PMNs are recruited into the lungs of animals infected by invasive aspergillosis and the absence of such recruitment leads to uncontrolled pulmonary fungal invasion (Balloy et al., 2005). Phagocyte stimulation by granulocyte colony-stimulating factor dramatically improves survival following Candida albicans lethal challenge (van Spriel et al., 2001). The mechanism by which PMNs are recruited to sites of fungal infection is believed to involve a combination of chemokine and PMN surface receptors, an interaction which polarizes Th1 antifungal activity (Traynor et al., 2002). One such chemokine, RANTES (regulated on activation, normal T-cell expressed and secreted), has been shown to have a crucial role in trafficking and activating leukocytes towards sites of infection and inflammation (Schall 1990; Sozzani et al., 1997). RANTES adheres to inflamed vascular endothelium, causing accumulation of leukocytes (von Hundelshausen et al., 2001).

Non-haematological patients dying with severe sepsis have lower circulating RANTES concentrations compared to survivors (Cavaillon et al., 2003), suggesting a protective role for it; however, there is no information in patients with haematological malignancy. This patient group is characterized by profound thrombocytopenia and also has a high risk for IFI morbidity and mortality. Since platelets are a major source of RANTES, we investigated RANTES changes in such patients who had an IFI.

METHODS

Fourteen consecutive patients developing IFIs over a recent 12 month period were identified from admissions to Tawam Hospital’s Haematology Unit (a tertiary referral unit for the United Arab Emirates). Serum samples were collected on a daily basis (at 6:00 am) starting from the day before the first dose of chemotherapy, throughout the chemotherapy and period of myeloablation and until recovery or death. For those patients that developed an IFI, RANTES concentrations were determined on selected days: the day prior to chemotherapy, the first day of fungal infection diagnosis (significantly different from baseline; \( P = 0.001 \) and 9078 ± 2256 pg ml\(^{-1} \)) at recovery from the fungal infection (significantly different from lowest value; \( P < 0.0001 \)). Platelet counts were closely correlated with the RANTES levels \(( r = 0.63, P < 0.001 )\). The RANTES concentrations for the three patients who died were similar to those who survived at all equivalent timepoints, but were significantly lower at the time of death \((792 ± 877)\) compared to the values at recovery for survivors \(( P = 0.005 )\). The finding that patients who died from an invasive fungal infection had very low platelet counts and RANTES concentrations suggests that these could play a role in host response to such infections.
day of diagnosis of the IFI, throughout the IFI and on the day of recovery or death from the IFI. RANTES determinations were performed by a commercial ELISA kit (RANTES/CCL5 DuoSet cat no. DY278, R&D systems). The lower detection cut-off value was 16 pg ml\(^{-1}\).

Patients admitted with acute malignant haematological disease received standard induction chemotherapy according to international guidelines (Coiffier et al., 2002; Anonymous, 1992; Farag et al., 2005). Patients provided their informed written consent for this study, which was approved by the Tawam Hospital Research Review Committee.

The diagnosis of IFI was based on a modification of the EORTC/MSG (European Organisation for Research and Treatment of Cancer/Mycoses Study Group) criteria (Ascioglu et al., 2002). Patients who were treated empirically with systemic antifungal drugs were also included, i.e. patients with antibiotic-unresponsive neutropenic fever (AUNF). These had 72 h of neutropenic fever, negative blood cultures included, i.e. patients with antibiotic-unresponsive neutropenic fever were treated empirically with systemic antifungal drugs were also

At the onset of IFI diagnosis, treatment with liposomal amphotericin B at 5 mg kg\(^{-1}\) day\(^{-1}\) (3 mg kg\(^{-1}\) day\(^{-1}\) for empirical treatment) was commenced, increasing the dose up to 10 mg kg\(^{-1}\) day\(^{-1}\) depending on the response. Granulocyte colony-stimulating factors were given to all patients at the time of clinical onset of IFI.

Mean values of RANTES concentrations and platelet counts were compared by the Mann-Whitney test. The correlation between platelets and RANTES was determined by the Pearson r test. A P value of < 0.05 was considered significant.

**RESULTS AND DISCUSSION**

Invasive aspergillosis and candidosis in patients with haematological malignancy are associated with mortalities of around 60 % (Lin et al., 2001) and 40 % (Puzniak et al., 2004), respectively, even when effective antifungal antimicrobial agents are given. Recovery of quantity and function of neutrophils is a pivotal step in a successful outcome of an IFI, since few patients with persistent neutropenia and an IFI survive (Denning & Stevens, 1990). Recombinant colony-stimulating factors that increase the numbers of phagocytes and modulate their biological action are beneficial in treating IFI (Rowe, 1998). In addition, PMN recruitment, activation and trafficking to fungal infection sites, which is mediated by chemoattractant chemokines such as RANTES, are immunological responses that are critical for recovery from fungal infections in experimental models (Blease et al., 2000; Mehrad et al., 2000).

**Study patients and IFIs**

We recruited 14 patients with IFI. Ten of the 14 patients had acute myeloid leukaemia and were treated either with standard induction chemotherapy (nine patients) or with salvage therapy (one patient). Another four patients were diagnosed with acute lymphoblastic leukaemia and received appropriate induction chemotherapy.

Of the 14 patients, nine had invasive pulmonary aspergillosis (IPA), one had invasive sinus aspergillosis, one had candidemia, one had candidal pneumonia and two had antibiotic-unresponsive neutropenic fever. Eleven patients survived the IFI and three died from the IFI. Details of these patients are shown in Table 1.

**Serum RANTES concentrations**

In the 11 patients who survived the IFI, the mean ± SD (95 % confidence interval) RANTES level on the day prior to receiving chemotherapy was 7656 ± 877 (6078 to 9235) pg ml\(^{-1}\) (Fig. 1). The RANTES level progressively fell between baseline and the first day of onset of the IFI, when the level was 3723 ± 2443 (2081 to 5365) pg ml\(^{-1}\) (significantly different from baseline; \(P = 0.0001\)). RANTES levels fell further during the course of the fungal infection. The mean minimal daily value recorded during the IFI was 1159 ± 2242 (−347 to 2666) pg ml\(^{-1}\) (significantly different from the start of the IFI; \(P = 0.005\)). At recovery from infection the mean RANTES concentration was 9078 ± 2256 (7562 to 10 594) pg ml\(^{-1}\) (significantly different from the minimum; \(P < 0.0001\)). In the three patients who died from their IFI the corresponding levels (in pg ml\(^{-1}\)) were: baseline, 8422 ± 2573 (2028 to 14 816); start, 1608 ± 1463 (−2027 to 5244); lowest, 16; and death, 792 ± 877 (−1387 to 2972). The values at recovery for the survivors were significantly higher compared to those at death for those who died (\(P = 0.005\) (Table 1, Fig. 1).
Table 1. Patient characteristics, outcomes and RANTES concentrations, and platelet and neutrophil counts at start of chemotherapy (base), IFI onset (start), nadir and at recovery or death

Disease refers to haematological diagnosis and treatment. ALL, acute lymphoblastic leukaemia; AMB, liposomal amphotericin B; AML, acute myeloid leukaemia; AUNF, antibiotic unresponsive neutropenic fever; Def, definite; HSC, hepatosplenic candidiasis; Ind, induction chemotherapy; IPA, invasive pulmonary aspergillosis; ISA, invasive sinus aspergillosis; Poss, possible; Prob, probable; Salv, salvage chemotherapy.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>IFI</th>
<th>IFI diagnostic category</th>
<th>Disease and treatment</th>
<th>RANTES concentration (pg ml(^{-1}))</th>
<th>10(^{-9}) × Platelet count (l(^{-1}))</th>
<th>10(^{-6}) × PMN cell count (l(^{-1}))</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>IPA</td>
<td>Prob</td>
<td>AML, Ind</td>
<td>Base 11000 Start 3752 Nadir 16 Recovery/ death 10100</td>
<td>Base 60 Start 12 Nadir 12 Recovery/ death 26</td>
<td>Base 13600 Start 150 Nadir 150 Recovery/ death 2410</td>
<td>Survived</td>
</tr>
<tr>
<td>5</td>
<td>IPA</td>
<td>Prob</td>
<td>AML, Ind</td>
<td>Base 10250 Start 7957 Nadir 16 Recovery/ death 9911</td>
<td>Base 191 Start 125 Nadir 5 Recovery/ death 83</td>
<td>Base 4720 Start 3080 Nadir 20 Recovery/ death 220</td>
<td>Survived</td>
</tr>
<tr>
<td>6</td>
<td>IPA</td>
<td>Prob</td>
<td>AML, Ind</td>
<td>Base 4174 Start 3138 Nadir 16 Recovery/ death 1609</td>
<td>Base 17 Start 28 Nadir 24 Recovery/ death 605</td>
<td>Base 1000 Start 20 Nadir 20 Recovery/ death 8000</td>
<td>Survived</td>
</tr>
<tr>
<td>11</td>
<td>C. tropicalis</td>
<td>Def</td>
<td>AML, Ind</td>
<td>Base 9418 Start 16 Nadir 16 Recovery/ death 619</td>
<td>Base 47 Start 13 Nadir 4 Recovery/ death 13</td>
<td>Base 160 Start 100 Nadir 100 Recovery/ death 20</td>
<td>Died</td>
</tr>
<tr>
<td>12</td>
<td>Non-C. albicans</td>
<td>Poss*</td>
<td>AML, Ind</td>
<td>Base 10350 Start 2989 Nadir 16 Recovery/ death 16</td>
<td>Base 409 Start 9 Nadir 10 Recovery/ death 15</td>
<td>Base 772 Start 20 Nadir 20 Recovery/ death 200</td>
<td>Died</td>
</tr>
<tr>
<td>14</td>
<td>AUNF</td>
<td>Poss</td>
<td>AML, Ind</td>
<td>Base 6100 Start 895 Nadir 319 Recovery/ death 9800</td>
<td>Base 46 Start 9 Nadir 6 Recovery/ death 166</td>
<td>Base 200 Start 20 Nadir 20 Recovery/ death 5000</td>
<td>Survived</td>
</tr>
</tbody>
</table>

*Diagnosis based on bilateral pneumonia without halo signs or other feature of IPA in a patient with haemoptysis and severe mucositis, who was persistently neutropenic and on antibiotics, and had negative blood and broncho-alveolar bacterial cultures and positive broncho-alveolar lavage for Candida species.
Therefore, these results show that chemotherapy results in a progressive fall in serum RANTES concentrations, beginning on the second day of treatment, associated with myeloablative thrombocytopenia and leukopenia. At the beginning of an IFI, RANTES levels fell substantially in all except one patient, reaching concentrations that were approximately 40% of baseline values. These decreased further in all but one patient during the IFI, reaching minimum levels of only 10% of baseline. Subsequently, patients who recovered from their IFI had an associated recovery of RANTES levels to at least baseline concentrations. However, patients who died of the IFI showed either no recovery from minimal levels observed during the IFI (one patient) or only partial low concentration recovery (two patients).

Although the number of patients in this study is small, there is consistency in the pattern of RANTES changes for each patient with an IFI. These observations suggest that our findings are accurate.

**Platelet counts**

Platelet counts changed in a parallel fashion to the RANTES concentrations; the correlation between platelets and RANTES was significant \((r = 0.63, P < 0.0001)\) (Table 1). Platelet count data were available at the recovery stage for 10 surviving patients, seven of whom had platelet counts that had recovered to \(> 83 \times 10^9 l^{-1}\). In all three patients who died from IFI the platelets did not rise to above \(26 \times 10^9 l^{-1}\).

The correlation that we observed between platelet counts and RANTES concentrations is consistent with other data that suggest that platelets are the major source of this chemokine (Cavaillon et al., 2003). In addition to providing a powerful leukocyte chemoattractant, platelets may also have direct antifungal activity, through a direct hyphal damaging effect (Christin et al., 1998). The role of platelets in protecting against IFI is consistent with the persistent thrombocytopenia observed in each of the three patients who died compared to the recovery of platelet counts seen in the survivors of IFI.

The temporary increases in RANTES concentrations observed on some days, e.g. days 9 and 11 in patient 6 (Fig. 2c), may have arisen from the platelet transfusions given for supportive therapy at those times. It is not possible to determine whether platelet-transfusion-associated RANTES are biologically active and hence whether platelet transfusions have any therapeutic role in managing IFI. It is also notable that in several patients RANTES recovery occurred prior to platelet recovery (Fig. 2c). This suggests not only that there is an additional source for RANTES, e.g. the recovering gut epithelium (Ellis, 2004), but also that the direct antifungal activity of platelets may be different from its indirect activity mediated via RANTES. In addition, the only patient who did not show a substantial fall in RANTES during the IFI (patient 4) might have been able to sustain a normal RANTES level as a result of less damage to extra-platelet sources as he was the only subject to receive a salvage chemotherapy regimen.

![Fig. 2.](image_url) Results of serum RANTES and neutrophil tests from patients 12 (a), 10 (b) and 6 (c). (a) Patient 12 died from candidal pneumonia. Note persistent neutropenia, RANTES depletion at start of infection and abortive rise in RANTES during infection. (b) Patient 10 developed and recovered from IPA. Note the rise in RANTES at start of IFI followed by recovery as infection resolves. (c) Patient 6 developed and recovered from IPA. Filled circles indicate the timing of platelet transfusions. Note the fall in RANTES, rise in RANTES following platelet transfusions and final recovery of RANTES and platelets with recovery from IPA. Symbols: ■, RANTES; ×, neutrophils; ▼, platelets. Arrows indicate start and end of infection.
**Neutrophil counts**

Among the 11 survivors, the time at which neutrophils began to recover (defined as the first day of progressive rise in neutrophils above $0.1 \times 10^9 \text{l}^{-1}$) was the same as the time of RANTES recovery (defined as the first day of progressive rise in RANTES from the lowest level) in four patients, preceded RANTES recovery by at least 2 days in five patients and lagged behind RANTES recovery by at least 2 days in two patients. The neutrophil count remained $<0.1 \times 10^9 \text{l}^{-1}$ in the three patients who died.

The increased RANTES concentrations seen prior to adequate circulating leukocytes suggest that RANTES recovery may be an immunological prerequisite for activating and trafficking neutrophils to fungal infection sites.

**Selected patient examples**

Fig. 2 illustrates some notable findings. Fig. 2(a) shows the sequence of events in patient 12, who died from pneumonia caused by species other than *C. albicans*. RANTES levels fell from a baseline value of 10 350 pg ml$^{-1}$ to values that were generally below 2000 pg ml$^{-1}$ during the fungal infection. Temporary elevations of RANTES concentrations were noted during the last 7 days, some of which reached approximately baseline values, but were not sustained. They fell dramatically in the last 2 days prior to death. All three patients who died had similar sequential changes in RANTES.

Fig. 2(b) shows the results from patient 8, who had IPA. RANTES levels fell as described for patient 12, but substantial sustained recovery of RANTES was observed by day 15. Neutrophil recovery was seen to lag behind the RANTES recovery by approximately 2 days. Fig. 2(c) illustrates profound thrombocytopenia, neutropenia and severe depletion of RANTES at the time of IPA, as seen in patient 6. Recovery of RANTES levels occurred prior to platelet and neutrophil recovery. Platelet transfusions are associated with unsustained elevations of RANTES concentrations.

**Conclusions**

Our preliminary observations do not permit us to precisely determine the role that RANTES may have in governing the outcome of an IFI or to distinguish the individual contributions of neutropenia and thrombocytopenia. However, the finding of a substantially depleted RANTES environment in patients with IFI and of persistence of these low concentrations in patients who died from IFI requires further investigation, for example considering the potential antifungal therapeutic dual role that platelet transfusions may have and the effect of recombinant RANTES on the outcome of IFI.

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**REFERENCES**


