ELSEVIER

FEMS Immunology and Medical Microbiology 37 (2003) 45–51

FEMS Immunology and Medical Microbiology

www.fems-microbiology.org

Apotransferrin administration prevents growth of *Staphylococcus epidermidis* in serum of stem cell transplant patients by binding of free iron

Leni von Bonsdorff [a], Leila Sahlstedt [b], Freja Ebeling [a], Tapani Ruutu [b], Jaakko Parkkinen [a,*]

[a] *Finnish Red Cross Blood Transfusion Service, Kivihaantie 7, 00310 Helsinki, Finland*
[b] *Department of Medicine, Helsinki University Central Hospital, Helsinki, Finland*

Received 12 January 2003; received in revised form 25 February 2003; accepted 26 February 2003

First published online 10 April 2003

Abstract

We investigated the effect of free, non-transferrin-bound iron occurring in haematological stem cell transplant patients on growth of *Staphylococcus epidermidis* in serum in vitro, and prevention of bacterial growth by exogenous apotransferrin. *S. epidermidis* did not grow in normal serum at inoculated bacterial densities up to 10^3 cfu ml^{-1} but slow growth could be detected at higher initial inocula. Addition of free iron abolished the growth-inhibitory effect of serum, whereas addition of apotransferrin again restored it. Appearance of free iron and loss of growth inhibition coincided in patient serum samples taken daily during myeloablative therapy. Intravenously administered apotransferrin effectively bound free iron and restored the growth inhibition in patient sera. The results suggest that exogenous apotransferrin might protect stem cell transplant patients against infections by *S. epidermidis* and possibly other opportunistic pathogens.

Keywords: Apotransferrin; *Staphylococcus epidermidis*; Non-transferrin-bound iron; Transferrin saturation; Stem cell transplantation; Opportunistic bacterium

1. Introduction

Infections cause significant morbidity and mortality in haematological patients receiving myeloablative conditioning therapy and allogeneic stem cell transplantation [1,2]. *Staphylococcus epidermidis* is a major causative agent of bacteraemia and septic infections in neutropenic patients receiving intensive immunosuppressive therapy [3,4].

During chemotherapy-related neutropenia, plasma-based resistance factors against bacterial infections may become critical. One of the factors known to influence microbial growth is iron accessibility [5]. In normal conditions, plasma iron is tightly bound to the high-affinity iron carrier protein transferrin, and the level of redox-active, non-transferrin-bound iron (NTBI) in plasma is very low. This is considered to prevent the growth of opportunistic pathogens, which do not possess efficient molecular mechanisms to utilise transferrin-bound iron [6]. On the other hand, several studies have indicated that high-dose chemotherapy of haematological malignancies is associated with an increase in serum total iron content, which often exceeds the iron binding capacity of transferrin and results in the appearance of NTBI in the serum of the patients [7–10]. Complete transferrin saturation and presence of NTBI are particularly common during myeloablative therapy and stem cell transplantation [11–13].

There is both experimental and clinical evidence suggesting that hyperferraemia predisposes patients to infections with opportunistic pathogens. In experimental animal infections, the virulence of several organisms was markedly enhanced by iron administration to the host [14]. Several studies have found an association between fungal infections and a high transferrin saturation level in neutropenic leukaemia patients [15–18]. It has also been suggested that high transferrin saturation contributes to lethality of pneumococcal pneumonia [19]. However, it

* Corresponding author. Tel.: +358 (9) 5801262; Fax: +358 (9) 5801429.
E-mail address: jaakko.parkkinen@bts.redcross.fi (J. Parkkinen).

0928-8244/03/$22.00
doi:10.1016/S0928-8244(03)00109-3

is not known whether high transferrin saturation as such, or the presence of NTBI, promotes microbial virulence.

In the present study we investigated the influence of transferrin saturation and NTBI on the growth of *S. epidermidis* in serum. The role of iron uptake mechanisms of *S. epidermidis* in the serum milieu has been unclear. Early studies indicated that in contrast to *Staphylococcus aureus*, coagulase-negative staphylococci were not able to grow in normal serum without iron supplementation [20]. On the other hand, it has been demonstrated that both *S. epidermidis* cells and the siderophore staphyloferrin A can displace iron from transferrin under in vitro conditions [21]. In our earlier study in serum-free conditions, *S. epidermidis* was not able to utilise transferrin-bound iron for growth unless high bacterial densities were present [22]. In the present study we found that *S. epidermidis* was not able to grow in serum unless either NTBI or a high initial count of bacteria was present. Further, we demonstrated that it is possible to prevent both the appearance of NTBI and growth of *S. epidermidis* in the serum of stem cell transplant patients by giving repeated infusions of exogenous apotransferrin.

2. Materials and methods

2.1. Patients

Twenty-six patients with haematological malignancies receiving allogeneic stem cell transplantation after myeloablative conditioning with cyclophosphamide and total body irradiation were enrolled in clinical trials with different doses of an investigational human plasma apotransferrin product (Apotransferrin SPR, Finnish Red Cross Blood Transfusion Service) [23]. Six patients received a single intravenous dose of 100 mg kg^{-1} of apotransferrin on day 3 after the stem cell transplantation [24]. Twenty patients were given repeated doses daily or every second day starting 6 days before the stem cell transplantation and continuing for 14–18 days. The total dose per patient varied between 0.3 and 0.6 g kg^{-1} in a low-dose group of 12 patients and was 1 g kg^{-1} in a high-dose group of eight patients. Ten patients were from a previous follow-up study and did not receive apotransferrin [13]. Prophylactic antibacterial agents routinely given to the patients were sulfamethoxazole/trimethoprim or ciprofloxacin. The clinical trials were approved by the ethical committee of the Helsinki University Central Hospital. Informed written consent of the patients was obtained.

2.2. Bacterial strains and culture conditions

Two multi-resistant clinical isolates of *S. epidermidis* (16779 and 19435) from neutropenic patients, which were resistant to methicillin and at least two antibiotic classes other than β-lactams, and the ATCC 12228 strain were used. Siderophore production was induced by precultivation in iron-depleted RPMI 1640 medium as previously described [22]. The bacteria were harvested in the late exponential growth phase and 10-fold dilutions were prepared in physiological saline.

An inoculum of 50 μl was added to 200 μl of serum buffered with 50 mM HEPES buffer, pH 7.4, in microwells. The growth was monitored by optical density (OD) for 24 h at 37°C with periodic shaking (Bioscreen C Microbiology Reader, Labsystems, Finland) in an airtight chamber maintained in a 5% CO_2 atmosphere. Serum pH was maintained at 7.3–7.4 and bicarbonate level at about 17 mM throughout the culture period. The detection limit for the increase in OD in 24 h (ΔOD) was 0.05 and the threshold density of *S. epidermidis* which could be detected was about 10^6 cfu ml^{-1}. Viable counts were determined by plating on trypticase-soy agar plates (bioMérieux, France). Sera with in vitro additions of ferric nitrilotriacetic acid prepared as described in [22] or apotransferrin were preincubated for 30 min at 37°C in 3% CO_2 to ensure binding of iron to transferrin.

2.3. Transferrin, iron and other serum assays

Serum transferrin (reference range 1.75–3.13 g l^{-1}) and C-reactive protein (CRP) (reference range <10 mg l^{-1}) were measured by immunoturbidimetric methods. Serum total iron was measured by a colorimetric ferene-S method (reference range 8–30 μM for females and 10–31 μM for males). All above-mentioned measurements were done at the HUCH Laboratory Diagnostics of the Helsinki University Central Hospital. Transferrin saturation (%) was calculated by the formula: serum iron (μM)/serum transferrin (g l^{-1})×3.98. NTBI was measured using the bleomycin-detectable iron assay adopted for small (25 μl) serum volumes [25]. Briefly, bleomycin was added to serum in the presence of ascorbic acid. Redox-active iron bound to bleomycin caused degradation of DNA in the mixture and the DNA degradation products were measured in microwell plates by a colorimetric thiobarbituric acid assay. A standard curve with 0.1–3 μM pure iron in water was calculated by linear regression of logarithmic values. The assay had a detection limit of 0.1 μM. The pH and bicarbonate concentrations in serum were measured using a blood gas analyser (ABL700, Radiometer).

3. Results

3.1. Influence of NTBI on the growth of S. epidermidis in serum

No growth of *S. epidermidis* took place in normal serum samples inoculated with 10^3 cfu ml^{-1} of the bacteria, whereas the bacteria proliferated rapidly in serum supplemented with iron (Fig. 1A). The three studied strains dis-

played similar iron dependence in their growth. Normal serum samples had transferrin saturation of 24–30% and NTBI <0.1 μM. The amount of iron added to serum (45 μM) was sufficient to fully saturate transferrin and form NTBI in a concentration of 3.2 ± 0.8 μM (mean ± S.D., $n = 5$). When both apotransferrin (2 g l^{-1}) and iron were added to the serum, no growth of the bacteria was observed (data not shown).

The influence of NTBI on bacterial growth in patient serum was studied with the two multiple drug-resistant *S. epidermidis* strains. Both strains grew rapidly in the serum samples of the three patients studied, which all contained fully saturated transferrin and NTBI (0.3 ± 0.1 μM). The addition of 0.1 g l^{-1} apotransferrin, which bound the NTBI, fully prevented the growth of the staphylococci, whereas the addition of iron-saturated holotransferrin had no effect. The growth curves from one patient with and without transferrin additions are shown in Fig. 1B.

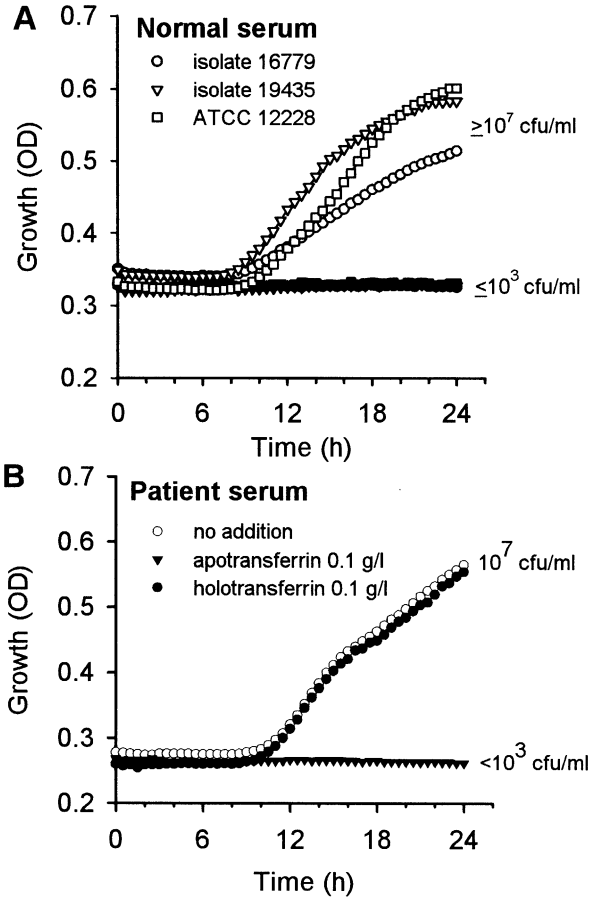

Fig. 1. Effect of in vitro addition of iron and transferrin on growth of *S. epidermidis* in serum. An inoculum of 10^3 cfu ml^{-1} was added to buffered serum, growth was carried out at 37°C in a 5% CO_2 atmosphere and monitored by optical density. Viable counts determined after 24 h are shown beside the growth curves. A: Two clinical isolates, 16779 and 19435, and the ATCC 12228 strain were grown in normal serum (closed symbols) and in the same serum with added NTBI (open symbols). B: Growth of strain 16779 in a patient serum containing 0.4 μM NTBI and in the same serum after addition of 0.1 g l^{-1} iron-free apotransferrin or 0.1 g l^{-1} iron-saturated holotransferrin.

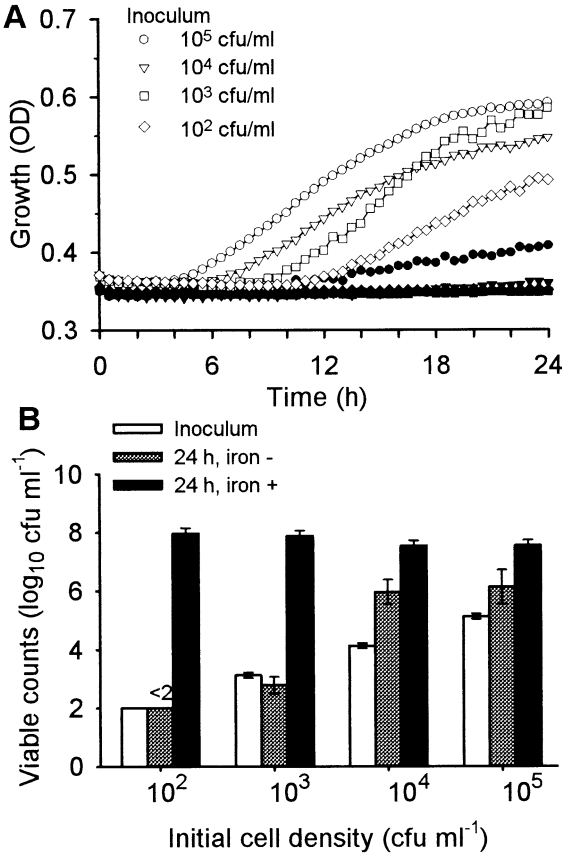

Fig. 2. Effect of the initial bacterial count on the growth of *S. epidermidis* in normal serum and in serum containing NTBI. A: Growth curves of different initial bacterial densities in normal serum (closed symbols) and in the same serum with added NTBI (open symbols). B: Viable counts at the beginning and after 24 h in the same sera. Growth of strain 16779 was monitored as described in Fig. 1. Bars indicate the mean and S.E.M. of repeated ($n = 4$) experiments. The difference between the viable counts after 24 h in normal serum and in the same serum with NTBI was statistically significant at all starting cell densities (10^3 cfu ml^{-1} $P < 0.0001$, 10^4 cfu ml^{-1} $P < 0.01$ and 10^5 cfu ml^{-1} $P < 0.05$, Student's *t*-test).

The influence of initial bacterial density on iron dependence of bacterial growth was studied in normal serum with and without iron addition. In iron-supplemented serum, inoculations in the range of 10^2–10^5 cfu ml^{-1} resulted in clearly detectable growth curves by OD monitoring, whereas without iron addition only the highest inoculum resulted in detectable growth (Fig. 2A). Determination of viable counts after 24 h indicated growth to 10^6 cfu ml^{-1} with starting densities of 10^4–10^5 cfu ml^{-1} and no detectable growth with 10^2–10^3 cfu ml^{-1}. After iron addition the growth consistently reached high concentrations (10^7–10^8 cfu ml^{-1}) with all inoculations (Fig. 2B).

3.2. Influence of apotransferrin administration on bacterial growth in patient serum

A bacterial inoculum of 10^3 cfu ml^{-1} was used in the microwell system with ΔOD measurement, which allowed identification of samples with growth to at least 10^6 cfu

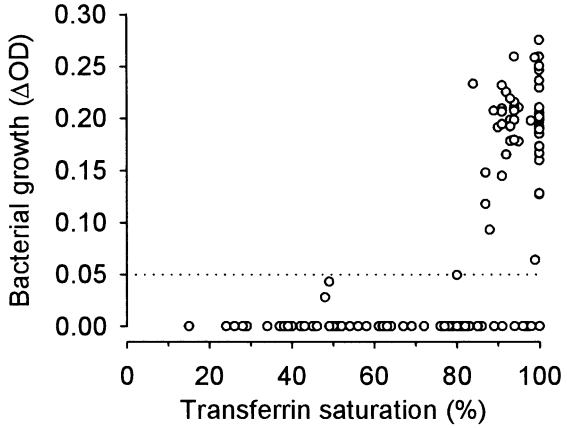

Fig. 3. Relation of bacterial growth measured as an increase in optical density (ΔOD) to transferrin saturation in the serum samples of the stem cell transplantation patients ($n = 132$). The growth of an inoculum of 10^3 cfu ml^{-1} of strain 16779 was monitored as the increase in optical density during 24 h. The line indicates the detection limit.

$ml^{-1} > 80\%$ (Fig. 3). Growth was detected in 87% (54/62) of the samples with NTBI, whereas only 4% (3/70) of the samples without NTBI grew ($P < 0.0001$, Fisher' $> 80\%$. Of the eight NTBI-positive samples not supporting growth, two were from a patient who had received vancomycin and four from a patient who was treated with a combination of tobramycin and ceftazidime.

In the first clinical trial in which a single intravenous dose of apotransferrin was given to the patients, serum samples from all six patients were positive for NTBI before apotransferrin administration and the inoculated bacteria grew in all of them (Fig. 4). Immediately after the administration, the growth was prevented concurrently with a drop in transferrin saturation and disappearance of NTBI. The length of this bacteriostatic effect of the single apotransferrin dose varied from 12 h to several days. The bacteriostatic effect typically disappeared simultaneously with the reappearance of NTBI in serum (Fig. 4). In the serum of a patient who received vancomycin on days 8–11, no growth of the staphylococci took place in spite of high transferrin saturation and NTBI on days 10–11 (Fig. 4B).

In subsequent trials, apotransferrin was administered as repeated doses starting 6 days before the stem cell transplantation on the same day as the conditioning therapy. For comparison, growth was monitored in two patients who did not receive apotransferrin (Fig. 5A). A sharp rise in serum iron content and appearance of NTBI occurred after the start of the conditioning and concomitantly, growth of inoculated staphylococci was detected. The lower apotransferrin doses tested were not sufficient to prevent the appearance of NTBI. The transferrin saturation remained at a high level, and bacteria grew in the serum samples which were positive for NTBI (Fig. 5B). Repeated higher doses prevented the appearance of NTBI in five of the eight patients studied. No significant bacterial growth could be detected in the serum of a patient with no detectable NTBI during the whole study period (Fig. 5C).

3.3. Infection markers in the patients receiving apotransferrin

To gain insight into whether NTBI is a risk factor for septic infections, markers for septic infections (fever $> 38° > 30$ mg l^{-1}) were compared between the 10 patients who did not receive apotransferrin, 12 patients who re-

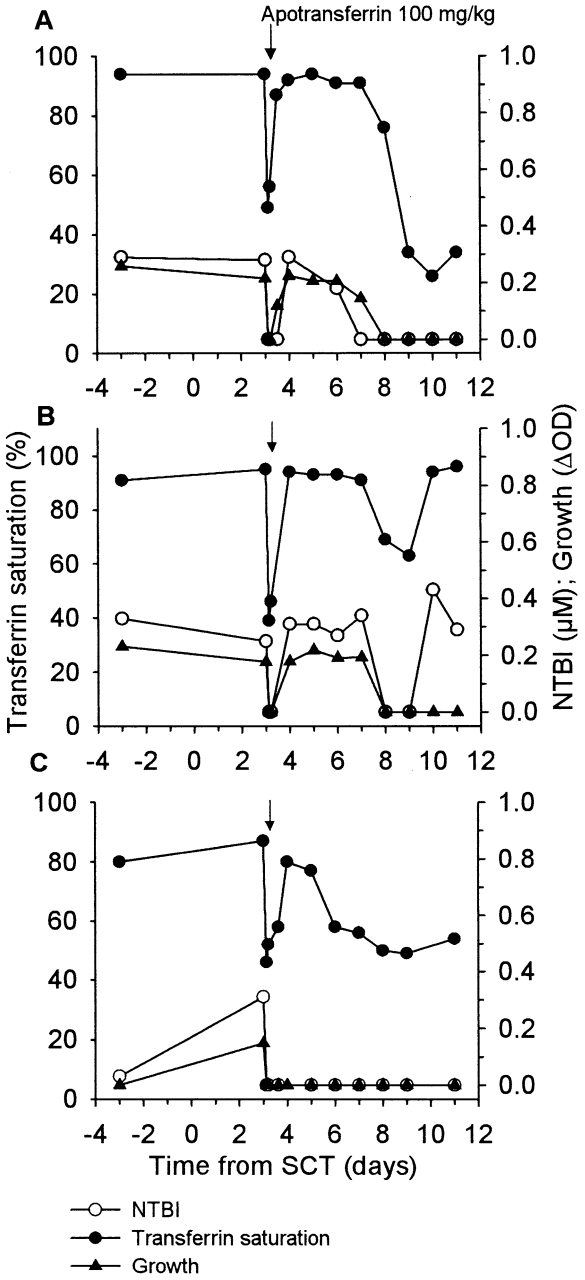

Fig. 4. Effect of a single intravenous administration of apotransferrin on transferrin saturation, NTBI and growth of *S. epidermidis* in the serum samples of three stem cell transplantation patients. Bacterial growth was measured as in Fig. 3. The arrow indicates the intravenous injection of 100 mg kg^{-1} apotransferrin 3 days after the stem cell transplantation.

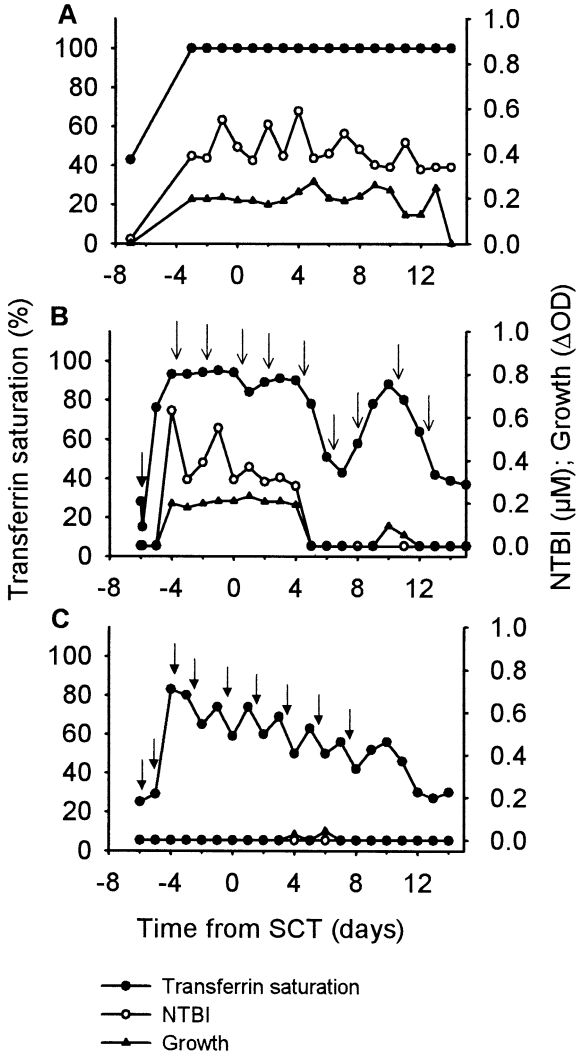

Fig. 5. Transferrin saturation, NTBI and bacterial growth in the serum of three stem cell transplantation patients, of whom one (A) did not receive apotransferrin and two (B,C) received repeated intravenous apotransferrin infusions at two different dose levels. Bacterial growth was monitored as in Fig. 3. Arrows indicate the apotransferrin infusions, closed and open arrows corresponding to doses of 100–115 mg kg^{-1} and 26 mg kg^{-1}, respectively.

ceived repeated lower doses of apotransferrin and eight patients who received repeated higher doses. Positive blood cultures were obtained only from a minority of patients with suspected septic infections, which probably was due to the use of prophylactic antibiotics and rapid onset of treatment with intravenous antibiotics when signs of septic infections were detected.

Most patients were positive for the infection markers at least on one occasion during the stem cell transplantation period. When the total number of days with positive infection markers was evaluated as a proportion of all study days, there was a significant reduction in the number of days with fever and days with elevated CRP in the patient groups who received low and high doses of apotransferrin, respectively (Table 1). When both markers were evaluated together, there was a significant reduction in the high-dose group ($P < 0.01$). The number of days with intravenous antibacterial treatment was significantly lower in both patient groups receiving apotransferrin than in the control group. There was also a trend towards a lower proportion of days with antifungal therapy in the higher-dose group but this was not statistically significant.

4. Discussion

The results of the present study show that at initial densities up to 10^3 cfu ml^{-1}, *S. epidermidis* was able to grow in serum only when the transferrin was fully saturated and NTBI was present. This was shown both by adding iron to normal serum and by studying serum samples of patients undergoing stem cell transplantation, in which transferrin became fully saturated and NTBI appeared after the initiation of the myeloablative therapy. Further, the growth of *S. epidermidis* in serum was effectively prevented when apotransferrin was added to the serum in vitro or when apotransferrin was intravenously administered to the patients to bind NTBI in circulation.

The results are in agreement with earlier findings [20], indicating that in contrast to *S. aureus*, coagulase-negative staphylococci could not grow in serum without iron supplementation. We conducted the bacterial culturing under conditions which kept serum bicarbonate and pH at physiological levels, both of which influence transferrin iron binding [26]. We found 80% to be a threshold for transferrin saturation, above which bacterial growth took place in patient samples. In a previous study, this was also identified as the critical saturation level, above which redox-active NTBI occurred in patients' sera [25]. In accordance, we observed a strong association between bacterial growth and NTBI in the patient serum samples.

The ability of *S. epidermidis* to multiply in serum in the absence of NTBI was dependent on the initial bacterial density. The bacteria grew in normal serum only at high initial densities (10^4–10^5 cfu ml^{-1}) and the growth was much slower than in the presence of NTBI. This is in line with our earlier findings in serum-free conditions showing that in the presence of purified transferrin the growth of *S. epidermidis* was critically dependent on full transferrin saturation and the presence of NTBI [22] > 10^5 cfu ml^{-1}). It has previously been demonstrated that *S. epidermidis* cells at high densities (10^8 cfu ml^{-1}) could displace iron from transferrin [21]. This was attributed to a cell wall transferrin binding protein, identified as glyceraldehyde-3-phosphate dehydrogenase. However, a more recent study on iron acquisition mechanisms of *S. aureus* showed that this glycolytic enzyme has no affinity for transferrin and another cell wall protein with iron-regulated expression was identified as a transferrin binding protein [27]. It is possible that multiple mechanisms, such as acidification and accumulation of chelating agents as a result of bacterial metabolism promote iron liberation

Table 1
Comparison of total number of days with positive infection markers and treatment with intravenous antibiotics between the patient groups who received no apotransferrin or received increasing total doses

Apotransferrin dose	NTBI-positive	Infection markers			Antibiotic therapy	Antifungal therapy
		$T > 38$°C	> 30 mg l^{-1}[a]	Both markers		
No apotransferrin	144/194	33/210	51/152	84/362	107/210	35/210
($n = 10$)	(74%)	(16%)	(33%)	(23%)	(51%)	(17%)
0.3–0.6 g kg^{-1}	102/252	20/252	53/158	73/410	87/252	44/252
($n = 12$)	(40%)**	(8%)*	(34%)	(18%)	(35%)**	(18%)
1.0 g kg^{-1}	32/168	16/168	22/102	38/270	51/168	16/168
($n = 8$)	(19%)**	(10%)	(22%)*	(14%)**	(31%)**	(10%)

NTBI was measured by the bleomycin assay.
Data are number of positive days/total days (%) during the study period from 6 days before stem cell transplantation to 14 days after it. P values were calculated by the Fisher's exact test. *$P < 0.05$, **$P < 0.01$ vs. no apotransferrin dose.
[a]CRP was measured on an average of 14 of the total 21 days, mainly after the stem cell transplantation.

from transferrin in the presence of high bacterial densities [22,27].

The number of invading bacteria evidently plays a role in the pathogenesis of septic infections. *S. epidermidis* often contaminates central venous catheters, where biofilm formation promotes the adherence and protection of the bacteria [28]. In stem cell transplant patients, the oral mucosa is another possible route of infection due to treatment-related mucositis [29]. In catheter infections, there is an association between the number of organisms retrieved from the catheter surface and the risk of infection, and infection occurs only when the number exceeds a certain quantitative threshold [30,31]. In a study of catheter-related bacteraemias, blood drawn from the catheters had up to 10^5 cfu ml^{-1} of coagulase-negative staphylococci, whereas peripheral blood samples had 10^1–10^2 cfu ml^{-1} [32]. Another study showed that the mean bacterial cell concentration in peripheral blood samples in blood stream infections caused by coagulase-negative staphylococci was 33 cfu ml^{-1} [33]. It may be reasoned that a high local density of *S. epidermidis* in the intravenous catheters promotes bacterial growth also in the absence of NTBI, whereas the proliferation of low counts occurring in the peripheral blood may depend on the availability of NTBI.

In the present study, apotransferrin administration to the patients effectively bound NTBI and restored the growth-inhibitory effect of patient serum against *S. epidermidis*. Repeated higher doses of apotransferrin maintained the growth-inhibitory effect in the serum of five out of the eight patients during the whole stem cell transplantation period. Earlier studies have described the reduced ability of serum from leukaemia patients to prevent growth of *Pseudomonas aeruginosa* [34], *Escherichia coli*, *S. aureus* [35] and *Candida albicans* [15]. Several studies have also shown that transferrin displays a growth-inhibitory effect in vitro on opportunistic organisms, such as *Vibrio vulnificus* [36], *Rhizopus* [37], *C. albicans* [15,38] and *Histoplasma capsulatum* [39]. Recently, an anticryptococcal protein in serum was identified as apotransferrin [40]. Thus, apotransferrin administration could have an impact on infections caused by several bacteria or fungi.

Among the small number of patients in the present study, the incidence of septic infections was not significantly different in the patients who received apotransferrin than in the control patients. When the patient groups were compared with respect to the total number of days with fever or elevated CRP, the patients who received repeated doses of apotransferrin had significantly fewer positive days than the patients who did not receive apotransferrin. Correspondingly, the number of days with intravenous antibiotics was significantly smaller during the study period in the patients who received repeated apotransferrin infusions. Although the patients were not compared in a randomised setting, this may suggest that repeated infusions of apotransferrin could protect the neutropenic patients against septic infections.

In conclusion, our results suggest a major pathogenic role for NTBI in septic *S. epidermidis* infections in neutropenic patients. With increasing resistance of microbes to antibiotics, binding of NTBI with apotransferrin could offer a method to enhance physiological resistance against infections. However, the clinical efficacy of this therapeutic concept remains to be investigated in prospective controlled trials.

Acknowledgements

We thank Mrs S. Lehmonen, Mrs A. Remes and Mrs T. Kupari for expert technical assistance.

References

[1] Walter, E.A. and Bowden, R.A. (1995) Infection in the bone marrow transplant recipient. Infect. Dis. Clin. N. Am. 9, 823–847.
[2] Elishoov, H., Or, R., Strauss, N. and Engelhard, D. (1998) Nosocomial colonization, septicemia, and Hickman/Broviac catheter-related infections in bone marrow transplant recipients. A 5-year prospective study. Medicine (Baltimore) 77, 83–101.
[3] Wade, J.C., Schimpff, S.C., Newman, K.A. and Wiernik, P.H. (1982) *Staphylococcus epidermidis*: an increasing cause of infection in patients with granulocytopenia. Ann. Intern. Med. 97, 503–508.

[4] Winston, D.J., Dudnick, D.V., Chapin, M., Ho, W.G., Gale, R.P. and Martin, W.J. (1983) Coagulase-negative staphylococcal bacteremia in patients receiving immunosuppressive therapy. Arch. Intern. Med. 143, 32–36.
[5] Weinberg, E.D. (1984) Iron withholding: a defense against infection and neoplasia. Physiol. Rev. 64, 65–102.
[6] Ratledge, C. and Dover, L.G. (2000) Iron metabolism in pathogenic bacteria. Annu. Rev. Microbiol. 54, 881–941.
[7] Halliwell, B., Aruoma, O.I., Mufti, G. and Bomford, A. (1988) Bleomycin-detectable iron in serum from leukaemic patients before and after chemotherapy. Therapeutic implications for treatment with oxidant-generating drugs. FEBS Lett. 241, 202–204.
[8] Gordeuk, V.R. and Brittenham, G.M. (1992) Bleomycin-reactive iron in patients with acute non-lymphocytic leukemia. FEBS Lett. 308, 4–6.
[9] Harrison, P., Marwah, S.S., Hughes, R.T. and Bareford, D. (1994) Non-transferrin bound iron and neutropenia after cytotoxic chemotherapy. J. Clin. Pathol. 47, 350–352.
[10] Carmine, T.C., Evans, P., Bruchelt, G., Evans, R., Handgretinger, R., Niethammer, D. and Halliwell, B. (1995) Presence of iron catalytic for free radical reactions in patients undergoing chemotherapy: implications for therapeutic management. Cancer Lett. 94, 219–226.
[11] Bradley, S.J., Gosriwitana, I., Srichairatanakool, S., Hider, R.C. and Porter, J.B. (1997) Non-transferrin-bound iron induced by myeloablative chemotherapy. Br. J. Haematol. 99, 337–343.
[12] Durken, M., Nielsen, P., Knobel, S., Finckh, B., Herrnring, C., Dresow, B., Kohlschutter, B., Stockschlader, M., Kruger, W.H., Kohlschutter, A. and Zander, A.R. (1997) Nontransferrin-bound iron in serum of patients receiving bone marrow transplants. Free Radic. Biol. Med. 22, 1159–1163.
[13] Sahlstedt, L., Ebeling, F., von Bonsdorff, L., Parkkinen, J. and Ruutu, T. (2001) Non-transferrin-bound iron during allogeneic stem cell transplantation. Br. J. Haematol. 113, 836–838.
[14] Griffiths, E. (1999) Iron in biological systems. In: Iron and Infection. Molecular, Physiological and Clinical Aspects (Bullen, J.J. and Griffiths, E., Eds.), pp. 1–26. John Wiley and Sons, Chichester.
[15] Caroline, L., Rosner, F. and Kozinn, P.J. (1969) Elevated serum iron, low unbound transferrin and candidiasis in acute leukemia. Blood 34, 441–451.
[16] Maertens, J., Demuynck, H., Verbeken, E.K., Zachee, P., Verhoef, G.E., Vandenberghe, P. and Boogaerts, M.A. (1999) Mucormycosis in allogeneic bone marrow transplant recipients: report of five cases and review of the role of iron overload in the pathogenesis. Bone Marrow Transplant. 24, 307–312.
[17] Karp, J.E. and Merz, W.G. (1986) Association of reduced total iron binding capacity and fungal infections in leukemic granulocytopenic patients. J. Clin. Oncol. 4, 216–220.
[18] Iglesias-Osma, C., Gonzalez-Villaron, L., San Miguel, J.F., Caballero, M.D., Vazquez, L. and de Castro, S. (1995) Iron metabolism and fungal infections in patients with haematological malignancies. J. Clin. Pathol. 48, 223–225.
[19] Lambert, C.C. and Hunter, R.L. (1990) Low levels of unsaturated transferrin as a predictor of survival in pneumococcal pneumonia. Ann. Clin. Lab. Sci. 20, 140–146.
[20] Schade, A.L. (1963) Significance of serum iron for the growth, biological characteristics, and metabolism of *Staphylococcus aureus*. Biochem. Z. 338, 140–148.
[21] Modun, B., Evans, R.W., Joannou, C.L. and Williams, P. (1998) Receptor-mediated recognition and uptake of iron from human transferrin by *Staphylococcus aureus* and *Staphylococcus epidermidis*. Infect. Immun. 66, 3591–3596.
[22] Matinaho, S., von Bonsdorff, L., Rouhiainen, A., Lönnroth, M. and Parkkinen, J. (2001) Dependence of *Staphylococcus epidermidis* on non-transferrin-bound iron for growth. FEMS Microbiol. Lett. 196, 177–182.
[23] von Bonsdorff, L., Tölö, H., Lindeberg, E., Nyman, T., Harju, A. and Parkkinen, J. (2001) Development of a pharmaceutical apotransferrin product for iron binding therapy. Biologicals 29, 27–37.
[24] Sahlstedt, L., von Bonsdorff, L., Ebeling, F., Ruutu, T. and Parkkinen, J. (2002) Effective binding of free iron by a single intravenous dose of human apotransferrin in haematological stem cell transplant patients. Br. J. Haematol. 119, 547–553.
[25] von Bonsdorff, L., Lindeberg, E., Sahlstedt, L., Lehto, J. and Parkkinen, J. (2002) Bleomycin-detectable iron assay for non-transferrin-bound iron in hematologic malignancies. Clin. Chem. 48, 307–314.
[26] Harris, D.C. and Aisen, P. (1989) Physical chemistry of the transferrins. In: Iron Carriers and Iron Proteins (Loehr, T.M., Ed.), pp. 239–371. VCH Publishers, New York.
[27] Taylor, J.M. and Heinrichs, D.E. (2002) Transferrin binding in *Staphylococcus aureus*: involvement of a cell wall-anchored protein. Mol. Microbiol. 43, 1603–1614.
[28] Kloos, W.E. and Bannerman, T.L. (1994) Update on clinical significance of coagulase-negative staphylococci. Clin. Microbiol. Rev. 7, 117–140.
[29] Kennedy, H.F., Morrison, D., Kaufmann, M.E., Jackson, M.S., Bagg, J., Gibson, B.E., Gemmell, C.G. and Michie, J.R. (2000) Origins of *Staphylococcus epidermidis* and *Streptococcus oralis* causing bacteraemia in a bone marrow transplant patient. J. Med. Microbiol. 49, 367–370.
[30] Raad, I. (1998) Intravascular-catheter-related infections. Lancet 351, 893–898.
[31] Sherertz, R.J., Raad, I.I., Belani, A., Koo, L.C., Rand, K.H., Pickett, D.L., Straub, S.A. and Fauerbach, L.L. (1990) Three-year experience with sonicated vascular catheter cultures in a clinical microbiology laboratory. J. Clin. Microbiol. 28, 76–82.
[32] Flynn, P.M., Shenep, J.L., Stokes, D.C. and Barrett, F.F. (1987) In situ management of confirmed central venous catheter-related bacteremia. Pediatr. Infect. Dis. J. 6, 729–734.
[33] Herwaldt, L.A., Geiss, M., Kao, C. and Pfaller, M.A. (1996) The positive predictive value of isolating coagulase-negative staphylococci from blood cultures. Clin. Infect. Dis. 22, 14–20.
[34] Hunter, R.L., Bennett, B., Towns, M. and Vogler, W.R. (1984) Transferrin in disease II: defects in the regulation of transferrin saturation with iron contribute to susceptibility to infection. Am. J. Clin. Pathol. 81, 748–753.
[35] Gordeuk, V.R., Brittenham, G.M., McLaren, G.D. and Spagnuolo, P.J. (1986) Hyperferremia in immunosuppressed patients with acute nonlymphocytic leukemia and the risk of infection. J. Lab. Clin. Med. 108, 466–472.
[36] Chart, H. and Griffiths, E. (1985) The availability of iron and the growth of *Vibrio vulnificus* in sera from patients with haemochromatosis. FEMS Microbiol. Lett. 26, 227–231.
[37] Boelaert, J.R., de Locht, M., Van Cutsem, J., Kerrels, V., Cantinieaux, B., Verdonck, A., Van Landuyt, H.W. and Schneider, Y.J. (1993) Mucormycosis during deferoxamine therapy is a siderophore-mediated infection. In vitro and in vivo animal studies. J. Clin. Invest. 91, 1979–1986.
[38] Shiraishi, A. and Arai, T. (1979) Antifungal activity of transferrin. Sabouraudia 17, 79–83.
[39] Sutcliffe, M.C., Savage, A.M. and Alford, R.H. (1980) Transferrin-dependent growth inhibition of yeast-phase *Histoplasma capsulatum* by human serum and lymph. J. Infect. Dis. 142, 209–219.
[40] Sridhar, S., Ahluwalia, M., Brummer, E. and Stevens, D.A. (2000) Characterization of an anticryptococcal protein isolated from human serum. Infect. Immun. 68, 3787–3791.