Changes in plasma fibronectin during allogeneic bone marrow transplantation

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SUMMARY Plasma fibronectin concentrations were measured serially in nine patients undergoing allogeneic bone marrow transplantation. Concentrations were reduced by conditioning treatment and episodes of bacterial infection. Acute graft versus host disease may exacerbate or prolong this process. Reduced plasma fibronectin concentrations impair the function of the mononuclear phagocyte system and the maintenance of capillary endothelial integrity and may thus contribute to the morbidity and mortality in patients undergoing bone marrow transplantation.

Plasma fibronectin is a major opsonin for cells of the mononuclear phagocyte system, facilitating the clearance of toxic particulate matter from the circulation.¹ The low concentrations found in many severely ill or injured patients contribute to the characteristic microcirculatory damage, particularly in the kidney and lungs, which is a leading cause of morbidity and mortality in such patients.² Depletion of plasma fibronectin is seen in patients with acute leukaemia³ and occurs in response to infection and the clearance of cellular debris after chemotherapy. Patients undergoing allogeneic bone marrow transplantation experience many toxic insults that might lead to depletion of plasma fibronectin—for example, destruction of tissue due to cytoreductive chemotherapy and radiotherapy, sepsis, and acute graft versus host disease. As the adverse effects of depletion of fibronectin can be reversed with replacement treatment⁴ the changes in plasma fibronectin concentrations during bone marrow transplantation warrant documentation.

Patients and methods

We studied nine consecutive patients undergoing allogeneic bone marrow transplantation for various disorders (Table). Citrated venous blood samples were taken before conditioning treatment was begun and about three times a week during the inpatient stay. Extra samples were taken in response to clinical events. Plasma was separated within three hours after collection and stored at -20°C . Batches were assayed by single radial immunodiffusion on 1% agarose supplemented by 3% polyethylene glycol using a rabbit antihuman fibronectin antiserum and standards supplied by Behringwerke, Marburg-Lahn, West Germany, as previously described.⁵

Plasma concentrations of the acute phase reactant protein C-reactive protein were measured on the same samples by radial immunodiffusion using antisera obtained from Seward Laboratories.

Pretransplant conditioning consisted of intraven-

Details of patients studied showing number of days before normal fibronectin concentrations were sustained

Case No	Age and sex	Diagnosis	Maximum grade acute graft versus host disease	No of days to sustained normal fibronectin concentration
1	28 (F)	Acute myeloid leukaemia, first CR	II	52
2	20 (F)	Acute lymphoblastic leukaemia, first CR	III	90
3	30 (F)	Acute lymphoblastic leukaemia, first CR	I	18
4	8 (F)	Acute myeloid leukaemia, first CR	II	>64
5	27 (M)	T cell acute lymphoblastic leukaemia, first CR	II	25
6	2 (F)	Congenital neutropenia	I	17
7	33 (M)	Chronic granulocytic leukaemia, chronic phase	I	42
8	7 (M)	T cell acute lymphoblastic leukaemia, first CR	II	20
9	14 (M)	T cell acute lymphoblastic leukaemia, first CR	II	50

CR = Complete remission

Accepted for publication 29 May 1985

ous cyclophosphamide 60 mg/kg four and three days before transplantation followed (except in case 6) by total body irradiation in three fractions of about 3.3 Gy (330 rads) two days before, one day before, and on the day of transplantation. Bone marrow was harvested from a histocompatible sibling on the day of transplantation, anticoagulated with heparin and acid citrate dextrose, and infused through a macro-aggregate filter within four hours of collection. All patients received cyclosporin A as prophylaxis against acute graft versus host disease.

Episodes of infection were defined by a positive culture or a combination of fever, strong clinical suspicion, and a C-reactive protein concentration greater than 40 mg/l.⁶ When possible the diagnosis of acute graft versus host disease was confirmed by biopsy of the appropriate organ.

Results

Fig. 1 shows the changes in plasma fibronectin and C-reactive protein concentrations (mean and range) for the whole group up to 35 days after bone marrow transplantation. At least three values were

Fig. 1 Plasma fibronectin and C-reactive protein concentrations (mean and range) in nine patients undergoing allogeneic bone marrow transplantation. Shaded area = normal (mean $\pm$ 2 SD) range of plasma fibronectin concentrations in 57 hospital outpatient controls.

Fig. 2 Serial plasma concentrations of fibronectin and C-reactive protein after bone marrow transplantation in two cases.

----- = lower limit of normal plasma fibronectin concentrations. GvHD = graft versus host disease.

recorded for each point shown (mean 5.96). Plasma fibronectin concentrations fell during conditioning, so that on the day of transplantation they were below normal in five of the nine patients. Some recovery was seen within the first few days after marrow infusion, only to be followed by a further appreciable fall from about day 8 as patients began to develop infective problems (mainly bacterial) at a time of severe neutropenia. This phase was accompanied by the expected high C-reactive protein concentrations. By day 21 most patients were showing a return of neutrophils to the peripheral blood, reflected in fewer episodes of bacterial infection and C-reactive protein concentrations between 0 and 20 mg/l. All of these patients, however, developed acute graft versus host disease of varying severity. Although numbers were too small for statistical evaluation, the severity of acute graft versus host disease and the time taken to achieve sustained—

that is, maintained for greater than five days—normal plasma fibronectin concentrations (Table) were apparently related. Patients with grade I acute graft versus host disease took a mean of 17.5 (SE 0.5) days and the higher grades 46.5 (10.2) days. One patient (case 4) never regained sustained normal concentrations before dying of disseminated aspergillosis and staphylococcal septicaemia on day 65.

Fig. 2 shows two typical examples of changes in plasma fibronectin and C-reactive protein concentrations. The upper panel of the Figure (case 7) shows a dramatic fall in the plasma fibronectin concentration coincident with septicaemia and acute renal failure. Recovery of the plasma fibronectin concentration was apparently delayed by episodes of acute graft versus host disease. The lower panel (case 4) shows a pronounced reduction in plasma fibronectin concentration during conditioning followed by repletion during the first week. By day 10 an episode of probable infection accompanied by grade II acute graft versus host disease and declining plasma fibronectin concentrations had occurred. Concentrations rose as the episode was controlled, only to fall once again as acute graft versus host disease recurred in the absence of infection.

Discussion

This study shows that most patients undergoing allogeneic bone marrow transplantation exhibit pronounced depletion of plasma fibronectin. Two thirds of our patients had subnormal concentrations during conditioning, presumably as fibronectin opsonised cellular debris. The second major stimulus to the consumption of fibronectin is bacterial infection during the phase of profound neutropenia. Thirty five days after bone marrow transplantation four of the nine patients still had subnormal concentrations, and one patient took 90 days to sustain normal plasma fibronectin concentrations. Although episodes of infection may produce low plasma fibronectin concentrations for up to three weeks in patients with acute leukaemia,³ our data suggest that the development of severe acute graft versus host disease may delay the return of plasma fibronectin concentrations to normal. Further studies are needed to clarify this. Poor nutritional state, as seen in many of our patients, may also be a cause of low plasma fibronectin concentrations⁷ and merits further investigation in such patients. Sustained low plasma fibronectin concentrations would be expected to increase susceptibility to infection⁷ and, perhaps more importantly, to reduce the ability of the patient to tolerate further infective or inflammatory insults without serious damage to vital

organs.

Reduced plasma fibronectin concentrations facilitate pulmonary endothelial damage in injured and infected patients⁸ and may perhaps be a factor in the predisposition of patients after bone marrow transplantation to interstitial pneumonitis. Interestingly, it has been suggested that one of the biological roles of C-reactive protein is to localise plasma fibronectin at sites of tissue damage.⁹ Furthermore, plasma fibronectin is believed to contribute to the maintenance of capillary endothelial integrity, and it would be interesting to study plasma fibronectin concentrations in patients with the "leaky capillary syndrome" which has been reported as a particular complication of "mismatch" transplants.¹⁰ We have shown previously that plasma exchange results in profound, if temporary, depletion of plasma fibronectin,¹¹ and hence the practice of performing intensive plasma exchange immediately before a major ABO group mismatch transplant might exacerbate the changes we observed. Recently, there has also been interest in depleting donor bone marrow of T lymphocytes to prevent acute graft versus host disease.¹² Low plasma fibronectin concentrations in the early period after transplantation will help frustrate strategies that rely on in vivo clearance of cells coated with monoclonal antibody by the patient's mononuclear phagocyte system.

It remains unclear whether replacement of plasma fibronectin would have an important effect on morbidity after bone marrow transplantation. A large controlled study would be needed to examine this issue in view of the complicated interplay of many toxic insults in these patients. The choice of a biologically active product rich in fibronectin also poses problems.¹³

We thank Dr JA Child, Dr DL Barnard, and Professor EH Cooper for much helpful advice and criticism during this study. Particular thanks are due to the nursing staff of the Bone Marrow Transplant Unit, St James's University Hospital, Leeds, for collecting the blood specimens.

This study was supported in part by the Yorkshire Regional Bone Marrow Transplant Fund.

The Yorkshire Regional Bone Marrow Transplant Group comprises: The Regional Radiotherapy Centre, Cookridge Hospital, Leeds; The Regional Blood Transfusion Centre, Leeds; The General Infirmary at Leeds; and St James's University Hospital, Leeds.

References

- 1 Simpson AW, Boughton BJ. Fibronectin as an opsonic regulator of monocyte phagocytosis. *J Clin Pathol* 1984;37:787-9.

- ² Saba TM, Jaffe EA. Plasma fibronectin (opsonic glyco-protein): its synthesis by vascular endothelial cells and role in cardiopulmonary integrity after trauma as related to reticuloendothelial function. *Am J Med* 1980;**68**:577-94.
- ³ Boughton BJ, Simpson A. Plasma fibronectin in acute leukaemia. *Br J Haematol* 1982;**51**:487-91.
- ⁴ Mosher DF, Grossman JE. Clinical use of fibronectin (potential applications). *Ric Clin Lab* 1983;**13**:43-54.
- ⁵ Norfolk DR, Bowen M, Roberts BE, Child JA. Plasma fibronectin in myeloproliferative disorders and chronic granulocytic leukaemia. *Br J Haematol* 1983;**55**:319-24.
- ⁶ Walker SA, Rogers TA, Riches PG, White S, Hobbs JR. Value of serum C-reactive protein measurement in the management of bone marrow transplant recipients. Part I: early transplant period. *J Clin Pathol* 1984;**37**:1018-21.
- ⁷ Scott RL, Sohmer PR, MacDonald MG. The effect of starvation and repletion on plasma fibronectin in man. *JAMA* 1982;**248**:2025-7.
- ⁸ Saba TM, Niehaus GD, Scovill WA, *et al.* Lung vascular permeability after reversal of fibronectin deficiency in septic sheep. Correlation with patient studies. *Ann Surg* 1983;**198**:654-62.
- ⁹ Salonen EM, Vartio T, Hedman K, Vaheri A. Binding of fibronectin by the acute phase reactant C-reactive protein. *J Biol Chem* 1984;**259**:1496-501.
- ¹⁰ Powles RL, Morgenstern G, McElwain TJ, *et al.* Mismatched family donors for bone marrow transplantation as treatment for acute leukaemia. *Lancet* 1983;ii:612-5.
- ¹¹ Norfolk DR, Bowen M, Cooper EH, Robinson EAE. Changes in plasma fibronectin during donor apheresis and therapeutic plasma exchange. *Br J Haematol* (in press).
- ¹² Prentice HG, Blacklock HA, Janossy G, *et al.* Use of anti-T cell monoclonal antibody OKT3 to prevent acute graft-versus-host disease in allogeneic bone marrow transplantation for acute leukaemia. *Lancet* 1982;ii:700-3.
- ¹³ Brown RA. Failure of fibronectin as an opsonin in the host defence system: a case of competitive self inhibition? *Lancet* 1983;ii:1058-60.

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