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Reduced function of the multidrug resistance P-glycoprotein in $CD34^+$ cells of patients with aplastic anaemia

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Summary. Drug exposure is implicated in the aetiology of some cases of acquired aplastic anaemia (AA), but the reason for this susceptibility remains unclear. We previously demonstrated that P-glycoprotein (P-gp) function, a drug efflux pump, is decreased in AA lymphocytes. To further evaluate whether P-gp activity is also abnormal in AA stem cells, we examined bone marrow (BM) $CD34^+$ cells from newly diagnosed AA patients (AA-d, $n = 25$), after immunosuppression (AA-IST, $n = 13$) and after BM transplantation (AA-BMT, $n = 8$). Of the AA patients with autologous haematopoiesis (AA-d + AA-IST), 15 had drug-induced AA. Thirty-two BM donors were studied as controls. P-glycoprotein function was assessed by the rhodamine 123-efflux assay. P-glycoprotein function in $CD34^+$ cells was reduced in AA-d patients (17·8%, 0–67·7) compared with controls (42·5%, 13·4–57·4; $P < 0·001$), as well as in AA-IST (20·3%, 1·2–32·0; $P < 0·001$), but not in AA-BMT (40·9%, 19·0–55·9). P-gp function was reduced more in drug-induced AA (14·5%, 0–27·4) than in the other cases (26·1%, 0–67·7; $P = 0·04$), but it did not correlate with disease severity. These results indicate that P-gp function is defective in AA $CD34^+$ cells, pointing to a role of P-gp in increased cell susceptibility to xenobiotics in AA.

Keywords: aplastic anaemia, stem cells, P-glycoprotein, multidrug resistance, rhodamine 123.

Aplastic anaemia (AA) is a life-threatening disease characterized by peripheral blood pancytopenia due to a hypocellular bone marrow (BM) (Young, 2000a). Acquired AA may be an idiosyncratic consequence of exposure to certain drugs, chemicals and viruses, although in the majority of cases no attributable cause is evident (Frickhofen *et al*, 1990; Kauffman *et al*, 1991). Consequently, immune-mediated damage to bone marrow stem cells has been proposed to be involved (Young, 2000b). According to this theory, these agents enter the haematopoietic cells, acting as haptens and generating neoantigens. These new peptides are recognized by antigen-presenting cells and then exposed to naïve T cells, which will proliferate and activate, establishing a type-1 cellular immune response dominated by T lymphocytes that both produce interferon-γ and are cytotoxic towards haematopoietic cells. Evidence of oligoclonal T-cell proliferation against $CD34^+$ cells was recently described in AA, but the responsible antigen(s) is still to be identified (Zeng *et al*, 2001). Fas-mediated apoptosis is the probable mechanism of cell death induced by immune destruction (Maciejewski *et al*, 1995; Callera & Falcão, 1997). However, the idiosyncratic nature of patient susceptibility to these agents is not understood, as the vast majority of exposed individuals do not develop AA (Young, 1999).

An explanation for the idiosyncrasy is that AA may be predetermined in some patients, and a possible mechanism of increased susceptibility could be an abnormal drug metabolism (Frickhofen *et al*, 1990). P-glycoprotein (P-gp), the *MDR1* gene product, is an energy-dependent transmembrane efflux pump for a variety of drugs and can cause multidrug resistance in neoplastic cells by actively extruding a wide range of structurally diverse, hydrophobic amphipathic substances from the cell (Higgins *et al*, 1997; McKenna & Padua, 1997). Furthermore, normal cells, such as haematopoietic cells, are known to express P-gp, whose physiological role is associated with cell protection against toxic compounds (Chaudhary & Roninson, 1991; Schinkel, 1997). Accordingly, the impairment of P-gp function causes cells to be susceptible to drug effects. The development of knockout mice for the *mdr1a* and *mdr1b* genes confirmed this hypothesis, resulting in viable and fertile animals, which, however, display increased sensitivity to drugs and altered pharmacokinetics (Schinkel *et al*, 1994, 1997).

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As the majority of drugs and chemicals involved in the aetiology of AA are hydrophobic, we have recently investigated whether P-gp activity is impaired in AA and found that it was decreased in peripheral blood T lymphocytes from AA patients (Calado *et al*, 1998). Interestingly, this altered activity was found not only in AA at presentation, but also during long-term remission after immunosuppressive therapy (IST), suggesting that this phenomenon might be intrinsic to these patients and might be responsible for the increased sensitivity to drugs observed in AA. However, P-gp activity in peripheral blood lymphocytes does not necessarily reflect its activity in haematopoietic stem cells, the injured cells in AA (Young, 2000a).

The aim of the present study was to investigate whether P-gp activity is also impaired in bone marrow CD34$^+$ cells of patients with AA by means of the flow cytometric rhodamine 123-efflux (Rh123) assay.

PATIENTS AND METHODS

Patients and controls. We studied BM samples from a total of 46 unselected patients with AA. Aplastic anaemia was diagnosed based on BM biopsy and peripheral blood counts, as recommended by the International Study of Aplastic Anaemia and Agranulocytosis (Kauffman *et al*, 1991). According to the criteria of Bacigalupo *et al* (1988), 11 cases were classified as moderate AA (mAA), 21 as severe AA (sAA) and 14 as very severe AA (vsAA). Three patients had hepatitis-associated AA and, in 17 cases, AA was associated with drug or chemical exposure, methimazole in one case, diclofenac in two, indomethacin in three, benzene-based solvents in two and pesticides in nine (carbamate-based pesticides in three and organophosphate-based pesticides in two; four patients did not remember the name of the pesticides used). Among these patients, 25 (aged 2–55 years, median 26 years) were analysed at diagnosis (AA-d) before any treatment was instituted (Table I). Thirteen patients (aged 12–67 years, median 29 years; Table II) were examined while in partial (transfusion independent) or complete remission after immunosuppressive therapy (AA-IST). The remaining eight patients (aged 12–57 years, median 19 years; Table III) were evaluated in remission after BM transplantation (AA-BMT). All patients in the AA-BMT group presented donor haematopoiesis, as assessed by cytogenetic or molecular analysis. Sequential samples from the same patient were excluded from analysis. Remission was established based on the criteria of Camitta (2000), and partial and complete responders were included in the remission groups (AA-IST and AA-BMT). All BM samples were collected after informed consent was obtained.

Table I. Clinical and laboratory data of patients with aplastic anaemia studied at diagnosis.

Patient Number	Age/Sex	Severity	Drug-induced	Haemoglobin (g/dl)	Neutrophils ($\times 10^9$/l)	Platelets ($\times 10^9$/l)
1	30/F	vsAA	No	7·2	0·1	47
2	12/F	vsAA	No	7·3	0·1	13
3	27/F	sAA	No	6·9	0·4	7
4	27/M	vsAA	No	4·9	0·1	20
5	2/F	sAA	Carbamate	5·1	0·3	5
6	4/F	vsAA	No	8·6	0·1	18
7	26/F	vsAA	No	8·2	0·1	5
8	30/F	vsAA	Methimazole*	4·7	0·1	5
9	7/F	sAA	No	7·5	0·5	5
10	25/F	sAA	No	6·4	0·3	3
11	16/F	vsAA	No	3·5	0·1	5
12	55/M	sAA	Carbamate	10·4	0·2	2
13	46/M	mAA	Pesticides	5·9	0·3	38
14	13/M	sAA	Benzene	7·8	0·3	11
15	17/M	vsAA	Benzene	6·0	0·1	9
16	30/M	sAA	No	6·7	0·3	11
17	12/M	sAA	Diclofenac	2·9	0·3	5
18	38/M	sAA	No	7·0	0·3	17
19	44/F	mAA	No	4·0	0·8	5
20	51/M	sAA	No	8·0	0·2	8
21	30/M	vsAA	No	9·8	0·1	36
22	18/F	sAA	No	6·7	0·3	7
23	55/F	vsAA	Indomethacin	2·6	0·1	3
24	24/F	mAA	No	9·3	2·9	4
25	17/M	sAA	No	10·6	0·7	6
Median (range)	26 (2–55)			6·9 (2·6–10·6)	0·3 (0·1–2·9)	7 (2–47)

*Hepatitis-associated aplastic anaemia.
mAA, moderate aplastic anaemia; sAA, severe aplastic anaemia; vsAA, very severe aplastic anaemia.

Table II. Characteristics of patients with aplastic anaemia in remission studied after immunosuppressive therapy: blood counts at time of study.

Patient Number	Age/Sex	Severity	Drug-induced	Haemoglobin (g/dl)	Neutrophils ($\times 10^9$/l)	Platelets ($\times 10^9$/l)
1	16/M	vsAA	Organophosphate	12·5	1·0	27
2	12/M	vsAA	No	11·6	0·9	79
3	29/M	mAA	No	12·6	4·5	63
4	67/F	sAA	No	11·0	1·1	23
5	59/F	mAA	No	11·3	1·7	38
6	28/M	sAA	Carbamate	10·2	0·9	20
7	44/F	vsAA	Pesticide	12·0	3·5	35
8	44/M	sAA	Pesticide	14·4	2·4	141
9	17/F	mAA	No	14·2	2·7	217
10	46/M	mAA	Organophosphate	15·5	3·4	121
11	29/M	mAA	No	15·9	1·9	84
12	29/M	sAA	Diclofenac	16·4	2·0	155
13	36/M	sAA	Pesticide	16·7	1·6	184
Median (range)	29 (12–67)			12·6 (10·2–16·7)	1·9 (0·9–4·5)	79 (20–217)

mAA, moderate aplastic anaemia; sAA, severe aplastic anaemia; vsAA, very severe aplastic anaemia.

Table III. Data of patients with aplastic anaemia studied after bone marrow transplantation. Haematological parameters at time of study.

Patient Number	Age/Sex	Severity	Drug-induced	Haemoglobin (g/dl)	Neutrophils ($\times 10^9$/l)	Platelets ($\times 10^9$/l)
1	30/M	sAA	No	18·7	2·2	172
2	19/F	vsAA	No	13·2	3·1	153
3	15/M	sAA	No	11·9	2·8	227
4	23/M	mAA	No*	13·9	2·7	181
5	12/F	sAA	No	14·1	2·0	227
6	14/F	sAA	Indomethacin	13·7	3·2	213
7	57/M	mAA	Indomethacin	14·7	5·3	151
8	19/M	mAA	No*	12·1	5·6	261
Median (range)	19 (12–57)			13·8 (11·9–18·7)	3·0 (2·0–5·6)	197 (151–261)

*Hepatitis-associated aplastic anaemia.
mAA, moderate aplastic anaemia; sAA, severe aplastic anaemia; vsAA, very severe aplastic anaemia.

Aplastic anaemia patients with autologous haematopoiesis (AA-d + AA-IST, $n = 38$) were further analysed and subdivided into patients with drug-induced AA ($n = 15$) and idiopathic AA ($n = 23$). Among the patients with drug-induced AA, association with methimazole was present in one case, non-steroidal anti-inflammatory drugs in three, solvents in two and pesticides in nine. These patients were also subdivided according to disease severity (mAA, $n = 8$; sAA, $n = 17$; and vsAA, $n = 13$).

Nine patients with other bone marrow failure syndromes (BMFS) were also studied. Among these patients, two were diagnosed as paroxysmal nocturnal haemoglobinuria (PNH), two as hypoplastic myelodysplastic syndrome, three as myelodysplastic syndrome (MDS) refractory anaemia type (MDS-RA) and two as MDS refractory anaemia with excess blasts type (MDS-RAEB). Four patients were men and five were women, aged from 18 to 78 years old (median, 46 years). Bone marrow samples from 31 healthy BM donors were collected and analysed as controls. Fifteen donors were males and 16 were females, aged from 10 to 64 years (median, 24 years), and their peripheral blood counts were in the normal range.

$CD34^+$ cell separation. Bone marrow samples were obtained by posterior iliac crest aspiration and stored in EDTA tubes, and BM mononuclear cells (BMMCs) were isolated by density gradient centrifugation. $CD34^+$ cells were purified from BMMCs by indirect-magnetic labelling (MACS, Miltenyi Biotec, Auburn, CA, USA), as described by

Killick *et al* (2000). Briefly, BMMCs were incubated with γ-globulin and hapten-conjugated anti-CD34 monoclonal antibody (clone QBEND/10) and then incubated with colloidal super-paramagnetic magnetic-activated cell sorter (MACS) microbeads conjugated to an anti-hapten antibody. Cells were washed and resuspended in Roswell Park Memorial Institute (RPMI)-1640 medium (Sigma, St. Louis, MO, USA) supplemented with 5% fetal calf serum to a final concentration of 5×10^6 cells/ml.

Rhodamine 123-efflux assay. P-glycoprotein activity was determined by means of Rh123 (Sigma) efflux, a fluorescent dye that is a substrate for P-gp, as previously described (Chaudhary & Roninson, 1991; Calado *et al*, 1998). Briefly, 200 μl of purified cell suspension were incubated with Rh123 (final concentration, 200 ng/ml) for 20 min. After washing, cells were incubated in a Rh123-free medium in the presence or absence of verapamil (Sigma), a P-gp inhibitor (final concentration, 10 μmol/l), for 1·5 h. Finally, cells were washed in a verapamil-containing RPMI medium and incubated with phycoerythrin-conjugated anti-CD34 (anti-HPCA-2, clone 8G12; Becton Dickinson, San Jose, CA, USA) monoclonal antibody and peridinin chlorophyll protein-conjugated anti-CD45 (anti-HLe-1, clone 2D1; Becton Dickinson), for 20 min. Up to 50 000 events were acquired and analysed in a FACScan flow cytometer (Becton Dickinson) equipped with an argon-ion laser with a wavelength setting of 488 nm. To investigate dye efflux in CD34$^+$CD45$^+$ cells, this cell subset was further identified and separated using multiple gating methods, according to the International Society of Hematotherapy and Graft Engineering (ISHAGE) protocol for enumeration of CD34$^+$ haematopoietic stem cells (Sutherland *et al*, 1996). Rh123 efflux was calculated based on the proportion of dye-effluxing cells in the verapamil-free experiment, in comparison to the control cells (treated with verapamil). Rh123 efflux was measured in CD34$^+$CD45$^+$ cells only.

Statistical methods. The Kruskal–Wallis non-parametric test followed by Dunn's multiple comparison test was used to compare P-gp function among multiple subgroups. The Mann–Whitney *U*-test was used to compare P-gp function between subgroups divided according to drug exposure and stem cell stigmata. A P value $< 0{\cdot}05$ was considered to be statistically significant.

RESULTS

CD34$^+$ cell enrichment

Separation of CD34$^+$ cells from BM samples by the immunomagnetic method yielded $5{\cdot}4 \pm 3{\cdot}1 \times 10^5$ cells in controls (CD34$^+$ purity, 74 ± 13·3%), $2{\cdot}6 \pm 0{\cdot}7 \times 10^5$ cells in patients with AA-d (CD34$^+$ purity, 7·1 ± 3%), $3{\cdot}3 \pm 2{\cdot}1 \times 10^5$ cells in AA-IST (CD34$^+$ purity, 44 ± 15%) and $4{\cdot}0 \pm 1{\cdot}0 \times 10^5$ cells in AA-BMT (CD34$^+$ purity, 63 ± 15%). Although CD34$^+$ cell purity did not reach high levels in samples from AA-d and AA-IST groups, it did not affect Rh123-efflux analysis, as this parameter was investigated in gated CD34$^+$CD45$^+$ cells only.

P-gp function in AA patients and controls

The Rh123-efflux assay was used to evaluate P-gp activity in CD34$^+$ cells from controls and patients with AA divided into three groups: AA-d, AA-IST and AA-BMT (Fig 1). Rhodamine 123 efflux revealed that P-gp function was significantly reduced in BM CD34$^+$ cells from AA-d patients (median, 17·8%; range, 0–67·7) compared with controls (42·5%, 13·4–57·4; $P < 0{\cdot}001$; Fig 2). Likewise, a similar

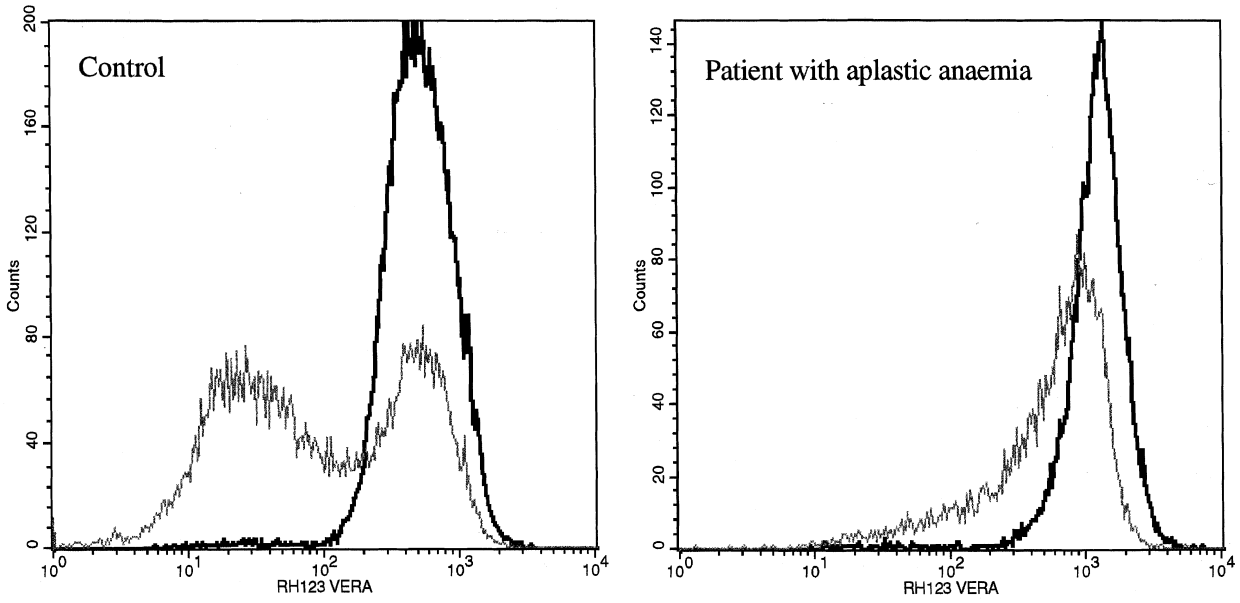

Fig 1. Rhodamine 123 efflux in bone marrow CD34$^+$ cells from a normal control and from a patient with aplastic anaemia after a 1·5 h efflux period in the presence (black line) or absence (grey line) of verapamil, a P-glycoprotein inhibitor.

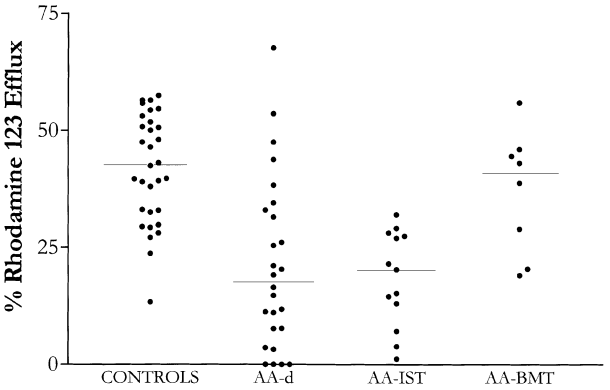

Fig 2. P-glycoprotein function in controls and patients. P-glycoprotein function is expressed as the percentage of rhodamine 123-effluxing bone marrow $CD34^+$ cells of controls, aplastic anaemia patients at diagnosis (AA-d, $n = 26$), aplastic anaemia patients in remission after immunosuppressive therapy (AA-IST, $n = 13$) and aplastic anaemia patients in remission after bone marrow transplantation (AA-BMT, $n = 8$). Patients from the AA-d and AA-IST groups presented significantly reduced P-glycoprotein function compared with controls ($P < 0{\cdot}001$). Bars represent the median of each group.

result was observed in AA-IST patients (20·3%, 1·2–32; $P < 0{\cdot}001$). However, P-gp function did not differ between normal controls and AA-BMT patients (40·9%, 19–55·9).

P-gp function versus drug exposure and disease severity

Aplastic anaemia patients with autologous haematopoiesis (AA-d + AA-IST) were further analysed to examine whether abnormally reduced P-gp activity correlated with exposure to xenobiotics and disease severity. P-gp function in BM $CD34^+$ cells was more noticeably reduced among drug-induced AA patients (14·5%, 0–27·4) than among idiosyncratic AA (26·1%, 0–67·7; $P = 0{\cdot}04$; Fig 3). However, P-gp function was independent of disease severity

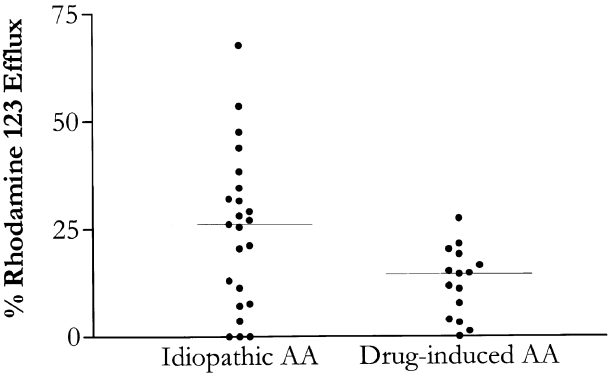

Fig 3. P-glycoprotein and the aetiology of aplastic anaemia. P-glycoprotein function is expressed as the percentage of rhodamine 123-effluxing bone marrow $CD34^+$ cells of idiopathic ($n = 23$) and drug-induced aplastic anaemia patients ($n = 15$). P-glycoprotein function was more markedly reduced in drug-induced than in idiopathic aplastic anaemia ($P < 0{\cdot}04$). Bars represent the median for each group.

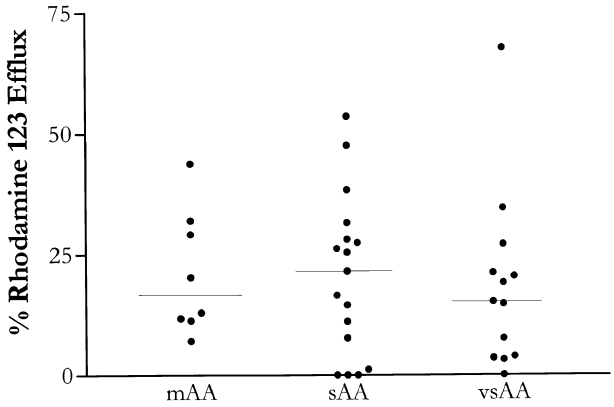

Fig 4. P-glycoprotein and disease severity. P-glycoprotein function is expressed as the percentage of rhodamine 123-effluxing bone marrow $CD34^+$ cells from patients with moderate (mAA, $n = 8$), severe (sAA, $n = 17$) and very severe aplastic anaemia (vsAA, $n = 13$). P-glycoprotein function was similar in the three groups. Bars represent the median for each group.

(mAA, 16·7%, 7·1–43·8; sAA, 21·5%, 0–53·6; and vsAA, 15·2%, 0–67·7), as illustrated in Fig 4.

P-gp function and stigmata of stem cell disease in aplastic anaemia

In order to investigate whether reduced P-gp function correlated with the stigmata of stem cell disease in AA patients in remission after IST, we analysed the association of P-gp functional expression and dysplastic bone marrow morphology, macrocytosis, thrombocytopenia and the presence of a paroxysmal nocturnal haemoglobinuria (PNH) clone. P-glycoprotein activity was similar in patients with or without the stigmata of stem cell injury (data not shown). However, P-gp function tended to be reduced more among patients in remission with persistent thrombocytopenia (platelet count $< 50 \times 10^9$/l) than in the other patients in remission, but the difference did not reach statistical significance ($P = 0{\cdot}09$).

P-gp function in other bone marrow failure syndromes

Nine patients with other bone marrow failure syndromes (BMFS) were evaluated in order to address whether decreased P-gp functional expression is also involved in the pathophysiology of other BMFS. Although lower levels of P-gp activity were found in this group of patients (29·4%, 2·9–54·7) in comparison with controls (42·5%, 13·4–57·4), the difference was not statistically significant.

DISCUSSION

Some cases of AA are known to be an idiosyncratic consequence of exposure to drugs and chemicals, however, the mechanisms involved in bone marrow failure triggered by these agents remain unclear (Frickhofen *et al*, 1990; Young, 1999). The cause of increased individual susceptibility may be related to abnormal drug metabolism (Frickhofen *et al*, 1990). In this respect, mutations in the cytochrome P450 leading to absent or impaired enzyme

activity have been assessed in patients with AA, but the analysis of these polymorphisms failed to demonstrate any association between cytochrome P450 variants and AA (Marsh *et al*, 1999). However, metabolic pathways are abundant and complex, involving multiple enzymes and making appropriate analysis difficult.

In the present study, the function of P-gp, a transmembrane drug transporter encoded by the *MDR1* gene, was examined in patients with AA by the flow cytometric rhodamine 123-efflux assay. This test has been proven be a sensitive and specific tool in determining P-gp function and is preferred in the evaluation of P-gp, in contrast to the analysis of P-gp expression by monoclonal antibodies (Wuchter *et al*, 2000). P-glycoprotein activity was demonstrated to be significantly decreased in BM CD34$^+$ cells from AA patients with autologous haematopoiesis, but not after BMT (Fig 2). Decreased P-gp function was more significant in patients with drug-induced AA (Fig 3). These results correlate with our previous findings that P-gp function is reduced in peripheral blood T lymphocytes from patients with AA at initial presentation and after IST (Calado *et al*, 1998). Based on the P-gp role in protecting haematopoietic stem cells against hydrophobic drugs, these data suggest that abnormally lower P-gp activity might be responsible for increased sensitivity to drugs in AA, as many xenobiotics involved in the aetiology of this disorder are hydrophobic. These results also indicate that BMT reverses P-gp function to the normal range. Furthermore, reduced P-gp function in AA patients who have recovered after IST correlates with the fact that this group of patients continues to present signs of defective haematopoiesis after immunosuppression (Marsh *et al*, 1990).

In support of the hypothesis that abnormal P-gp function might mediate increased sensitivity to xenobiotics in AA, it has been demonstrated that the disruption of mouse *mdr1* genes leads to animals with increased sensitivity to drugs (Schinkel *et al*, 1994, 1997). In these studies, the knockout animals were viable, fertile and phenotypically normal, but displayed increased susceptibility to the side-effects of the pesticide ivermectin and the anti-neoplastic drug vinblastine, indicating that defective P-gp function increases drug toxicity. Moreover, these animals presented reduced elimination of digoxin, a cardiac glycoside that is also a P-gp substrate. Unfortunately, the investigators did not examine haematopoietic function and bone marrow cellularity.

In addition to protecting cells against xenobiotics, P-gp seems to play a role in organism protection against viral infection. P-glycoprotein is assumed to mediate cellular uptake of viral particles and virus production (Lee *et al*, 2000). In this respect, reduced P-gp function might increase viral damage to haematopoietic cells in virus-associated AA. However, only one patient with autologous haematopoiesis had hepatitis-associated AA in our series, precluding appropriate analysis. It is noteworthy, however, that P-gp function was almost absent in CD34$^+$ cells from this patient (3·2%).

On the other hand, P-gp function in AA CD34$^+$ cells was independent of disease severity (Fig 4). These results are in agreement with previous data, indicating that the prognosis of drug-induced AA is similar to that of idiopathic AA (Malkin *et al*, 1990).

However, the reason for impaired P-gp function in AA remains to be elucidated. Based on the present findings, we cannot fully determine whether decreased P-gp activity is the cause or a consequence of AA, but some evidence suggests that this decrease is an early phenomenon in AA. First, BM damage could be responsible for decreased P-gp function as a result of CD34$^+$ cell stress and selection, but the reversal of P-gp function to normal levels after BMT argues against this hypothesis. In addition, reduced P-gp function in AA patients who have recovered after IST indicates that abnormal P-gp activity in AA is not a consequence of a skewed CD34$^+$ population. Second, the reduced P-gp function in recovered AA patients a long time after IST also suggests that this might be characteristic of CD34$^+$ cells from patients with AA. Third, the more markedly reduced P-gp activity in drug-induced AA in comparison to idiopathic cases further indicates that abnormal P-gp function might be involved in the aetiology of the disease. However, the rarity of this illness precludes prospective analysis of the risk of developing AA in individuals with decreased P-gp function. The study of drug effects on haematopoiesis in *mdr*-genes knockout animal models (Schinkel *et al*, 1994, 1997) may serve to address this question. Finally, we were not able to demonstrate an association between reduced P-gp functional expression and the stigmata of stem cell disease in AA, suggesting that P-gp malfunction is not a consequence of stem cell injury.

Moreover, we studied nine patients with other bone marrow failure syndromes (BMFS), such as PNH and MDS, in order to address whether reduced P-gp function is also present in other BMFS. However, we did not find significant P-gp function reduction among these patients. These data do not completely rule out the role of P-gp in the pathophysiology of these syndromes, as this group of patients was heterogeneous.

On the other hand, diminished P-gp function in AA might be environmentally and genetically determined. P-glycoprotein-mediated transport of digoxin has been shown to be impaired by the concomitant administration of the anti-arrhythmic quinidine (Fromm *et al*, 1999). Furthermore, other investigators demonstrated that rifampin induces an increase in P-gp expression in duodenal epithelial cells (Greiner *et al*, 1999). In addition to the above-mentioned environmental factors, there is strong evidence that P-gp expression and function are genetically regulated. The recently described C3435T polymorphism in the human *MDR1* gene determines a reduced P-gp expression in duodenum and a lower P-gp function in CD56$^+$ natural killer cells (Hoffmeyer *et al*, 2000; Hitzl *et al*, 2001). Thus, these genetic determinants may be implicated in the loss of P-gp function observed in AA. However, this polymorphism does not influence P-gp activity in normal BM CD34$^+$ cells (Calado *et al*, 2002). It is conceivable to speculate that P-gp function in CD34$^+$ cells may be regulated by still unknown *MDR1* gene polymorphisms, which might be responsible for defective P-gp activity in AA.

In conclusion, these data demonstrate for the first time that BM CD34$^+$ cells from patients with AA present reduced P-gp function, more noticeably in drug-induced AA, pointing to a role of P-gp in the drug sensitivity observed in the

disease. However, the basis for this abnormally decreased P-gp function, whether genetic or environmentally determined, remains to be elucidated.

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