Myeloid surface antigen abnormalities in myelodysplasia: relation to prognosis and modification by 13-cis retinoic acid

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SUMMARY The relation between prognosis and lineage specific surface antigen expression on peripheral blood granulocytes and monocytes was studied using monoclonal antibodies and flow cytometry in 37 patients with myelodysplastic syndromes (MDS). Abnormalities in antigen expression were summarised as a score, and cases were divided into low (few abnormalities) and high (many abnormalities) groups. Survival was significantly worse in the "high" group (logrank $\chi^2 = 5.793$, $p = 0.016$), this group having a median survival of 31 weeks, compared with more than 67 weeks in the "low" group. No correlations were found between the score and any of the following: peripheral blood platelet and granulocyte count; FAB subtype; bone marrow blast cells and sideroblast count, or erythroid and myeloid progenitor growth. Antigen expression was also studied in six further cases of MDS before and after six weeks of treatment with 13-cis retinoic acid (CRA), 20 mg given orally, and a comparison was made with six untreated patients studied before and after a similar time interval. In the treated group 58% of initially abnormal measurements reverted to normal, compared with 24% in the untreated group. Five of the six treated patients showed a decrease in the score, whereas only two of the six improved in the untreated group. The data indicate that myeloid antigen expression is a useful indicator of prognosis in MDS, and that antigen expression may be affected by treatment.

The myelodysplastic syndromes (MDS) are a group of haemopoietic stem cell disorders with differing manifestations but a common tendency to evolve into acute non-lymphoid leukaemia.¹ The FAB group proposed a classification of these syndromes based on morphological appearances,² but it has been suggested that a biological classification of MDS, based on functional abnormalities, may be of greater value.³ Granulocyte function is abnormal in many of these patients, and granulocyte adhesion, superoxide generation, chemotaxis, phagocytosis and intracellular killing capacity are abnormal in 50%.⁴ We have previously reported both decreased myeloid-macrophage antigen expression and increased expression of aberrant antigens in about 50% of patients with MDS at the time of presentation.⁵ We now report the relation between myeloid-macrophage surface antigen abnormalities and prognosis, and the changes in antigen expression induced by treatment.

13-cis retinoic acid (CRA) inhibits the development of papillomas induced by carcinogens in mice⁶ and the malignant transformation of cultured cell lines.⁷

It stimulates differentiation of HL60 cells while suppressing proliferation,⁸ and induces the expression of myeloid specific surface antigens.⁹ It also inhibits the proliferation of both normal and leukaemic colony forming units—granulocyte—macrophage (CFU-GM)^{10 11} and those of patients with MDS.¹² Therapeutic studies of CRA in patients with MDS, however, have so far proved disappointing. A beneficial effect on blood counts and marrow morphology has been described in individual cases,^{13 14} though whether continuous treatment with doses that are well tolerated changes overall patient survival is not yet clear.¹⁵ We report the effect of therapeutic CRA on myeloid surface antigens.

Patients and methods

Forty three patients with MDS were studied (21 men and 22 women, with an age range of 29–86 years; mean 61.8). All fulfilled the diagnostic criteria of Bennett *et al*² as modified by May *et al*,¹⁶ and included 14 patients with refractory anaemia, 10 with sideroblastic anaemia, nine with refractory anaemia with

Table 1 Antigen expression on myelodysplastic peripheral blood granulocytes and monocytes before and after six weeks' treatment with 13-cis retinoic acid

	Granulocytes					Monocytes					Surface antigen score
Case code	82H1	82H6	FMC11	Mo2	FMC17	82H1	82H6	FMC11	Mo2	FMC17	
Treated cases:											
CF (0)*	51	79	77	7	34	12	12	15	6	38	40
	65	88	91	5	26	3	7	3	14	24	20
WS (0.88)*	78	90	87	7	50	24	19	35	55	62	40
	72	84	47	4	20	7	18	34	44	48	30
EV (0.82)*	75	76	5	4	65	11	10	6	8	38	30
	81	88	29	6	25	9	12	10	13	56	40
AG	21	10	21	2	11	12	12	15	9	75	60
	61	65	77	8	41	7	9	10	19	42	10
IP	21	40	24	3	3	12	21	16	7	8	70
	12	57	29	6	5	7	9	7	9	52	30
MW	69	83	32	4	11	31	10	28	41	58	20
	63	51	30	5	5	6	10	28	46	47	10
Untreated cases:											
DDy	7	8	14	—	—	35	49	75	24	52	75
	3	9	8	—	—	67	70	63	34	59	75
CN	84	84	69	6	42	13	13	16	19	66	30
	92	92	86	2	54	27	12	45	40	47	40
BG	64	67	45	4	5	9	9	23	23	78	10
	44	77	64	7	3	6	6	15	15	55	0
TT	70	70	—	—	—	2	8	26	46	76	0
	68	60	77	7	—	23	13	20	54	58	22
DMo	51	89	67	6	78	10	28	6	7	26	40
	12	25	24	4	54	5	5	4	3	13	60
SCou	86	81	65	4	24	12	12	24	24	56	30
	70	83	76	9	26	6	5	13	32	47	10
Normal range	43–93	50–97	47–85	2–11	2–11	2–11	1–11	3–28	14–71	34–77	

*Plasma CRA concentration ($\mu\text{mol/l}$) taken one to two hours after an alleged 20 mg dose.

excess blasts (RAEB), three with RAEB in transformation, three with chronic myelomonocytic leukaemia, and four with idiopathic macrocytosis. Two patients only received low dose ara-C six and 16 months before the study.

MYELOID ANTIGENS

The expression of granulocyte specific and monocyte specific antigens was studied on both granulocytes and monocytes in peripheral blood using monoclonal antibodies. The granulocyte specific monoclonal antibodies used were 82H1 and 82H6¹⁷ and FMC11.¹⁸ 82H1 and 82H6 are CD15 monoclonal antibodies and recognise the X hapten.^{19,20} FMC11 recognises an antigen expressed on metamyelocytes onward¹⁸ but has not been defined specifically. The monocyte specific monoclonal antibodies used were FMC17 and Mo2, which belong to the CDw14 cluster.²¹

Granulocytes and monocytes were separated from peripheral blood, and antibody binding was measured on each cell type by indirect immunofluorescence, as previously described.⁵ Stained cell suspensions were analysed in a FACS III cell sorter (Becton Dickinson) using a 4 watt argon

ion laser at 488 nm. In a few cases samples were inadequate for study. The resulting fluorescence histograms were analysed using an Apple II EC Europlus microcomputer and homemade software, which calculated the percentage of positive cells for each sample by comparing each test histogram with a control histogram, as previously described.⁵

The normal range of antigen expression of granulocytes and monocytes was determined from 16 age matched volunteers who were either healthy laboratory staff or patients about to undergo minor eye surgery but who were otherwise healthy and not taking drugs. There were nine men and seven women with an age range of 25-84 years (mean 61.5).⁵ Table 1 shows the range of normal expression of each monoclonal antibody.

EFFECT ON PROGNOSIS

For each of the 37 cases on whom sufficient data were available, the degree of abnormality of surface antigen expression was summarised as a surface antigen score. This was defined as:

$$\frac{\text{number of abnormal measurements}}{\text{total number of measurements}} \times 100$$

Table 2 Prognostic relevance of low and high surface antigen scores

Surface antigen score	No of cases	No of deaths	No of leukaemic transformations	Mean survival (weeks)	75% (first quartile) survival (weeks)
Low (< 30)	20	8	1	111	89
High (> 30)	17	12	7	65	16
Total	37	20	8		

and is a measure of the proportion of abnormally expressed antigens. Abnormal expression was defined if the calculated percentage positivity fell outside the normal range (table 1).⁵ Because up to five measurements were made on both granulocytes and monocytes, the denominator of the surface antigen score varied from 6–10 (no case had less than six measurements). All cases were followed up from the time of investigation until death, or for between 65 and 148 weeks.

ADDITIONAL INVESTIGATION

In each case peripheral blood counts were carried out by standard techniques and Jenner-Giemsa stained marrow film were examined, at least 400 marrow cells being counted in each case. Dyserythropoiesis was defined as a specific nuclear abnormality such as a megaloblastic appearance, binucleation, the presence of nuclear budding or Howell-Jolly bodies, or gaps in the nuclear membrane. On day 7 numbers of colony forming units-erythroid (CFU-E) were assayed by a modification of the method of Iscove *et al*²² and CFU-GM colony numbers on day 7 by the method of Bailey-Wood *et al*.²³ The Spearman rank correlation coefficient between these measurements and the surface antigen scores were calculated using the Biomedical Computer Programs P-series (BMDP) package.²⁴ The relation between the FAB subtype and the surface antigen score was analysed by the Kruskal-Wallis one way analysis of variance by ranks.

EFFECT OF CRA

In six cases antigen expression was studied before and after six weeks' treatment with CRA in a dose of 20 mg given orally each day. None of these cases was included in the analysis of the effect of surface antigen scores on prognosis because CRA may have influenced their survival time. Six similar but untreated cases were also studied before and after an interval of six weeks. In three of the treated cases serum CRA titres were assayed by Dr G Allen of Roche Laboratories, using the method of McLean *et al*.²⁵ Some of the cases treated with CRA had minor mucocutaneous or conjunctival side effects. No change in liver function test results or triglyceride concentrations was observed.

Results

PROGNOSTIC RELEVANCE OF SURFACE ANTIGEN SCORE

The median value of the score (SAS) in the 37 cases for whom sufficient data were available was 30%. Cases were divided into a low (< 30%) and a high (> 30%) group, and table 2 shows the proportion of deaths and leukaemic transformations in each group. The difference in the rate of leukaemic transformations between the two groups was highly significant ($p = 0.014$, Fisher's exact test). Table 2 also shows the mean survival times at 65–148 weeks and the 75% survival times for each group. The median survival was 31 weeks in the high scoring group, but was not reached at 67 weeks in the low scoring group. The figure shows Kaplan-Meier survival curves for each group and the significant difference in survival (logrank $\chi^2 = 5.793$, $p = 0.016$).

RELATION BETWEEN SURFACE ANTIGEN SCORE AND OTHER PERIPHERAL BLOOD AND BONE MARROW ABNORMALITIES

No correlation was found between the score and peripheral blood platelet or granulocyte count, FAB subtype, bone marrow blast cells, sideroblast count or erythroid and myeloid progenitor growths. A weak negative correlation was observed with the peripheral blood haemoglobin ($r_s = -0.44$, $p = 0.006$).

Figure Kaplan-Meier survival curves for groups with high and low surface antigen scores.

Myeloid surface antigen abnormalities in myelodysplasia

EFFECT OF CRA

The effect of CRA on granulocyte and monocyte specific antigen expression by granulocytes and monocytes in peripheral blood is shown in table 1. In the treated group 15 of the 26 measurements abnormal initially (58%) reverted to normal, compared with only four of 17 (24%) in the untreated group. In the treated group 5% of measurements became abnormal during the study, compared with 11% in the untreated group. Five of the six treated cases showed an improvement in the surface antigen score, while only two of the six untreated cases improved; a further case remained abnormal (DDy). In two of the three patients in whom serum CRA concentrations were measured those achieved were about $1 \mu\text{mol/l}$; in the third patient no CRA was detected, suggesting non-compliance.

Discussion

Peripheral blood granulocytes and macrophages in MDS may exhibit not only decreased expression of appropriate antigens, but also increased expression of inappropriate ones.⁵ Hokland *et al*²⁶ showed increased bone marrow mononuclear cell expression of My7 in some cases; this monoclonal antibody detects a CDw13 antigen.²¹ Such patients tended to evolve to acute myeloid leukaemia more rapidly and have shorter survival. The present data show that abnormal surface antigen expression, summarised as a score, is associated with poor survival in MDS. Those patients with high scores tended to survive less than one year, whereas those with low scores usually survived longer than 18 months (figure).

Surface membrane glycoproteins are important in phagocytic cell function.²⁷ Membrane glycoproteins are important in mediating neutrophil adhesion²⁸ and macrophage migration.²⁹ Moreover, the stimuli which normally activate production of oxygen radicals in phagocytes depend on glycoprotein membrane receptors.³⁰ The defective surface membrane glycoprotein expression of myelodysplastic phagocytes shown here may represent a more general glycoprotein deficiency on these cells, leading to less efficient phagocytosis, increased susceptibility to serious infection, and decreased survival. Further work is required to relate abnormal phagocyte function to surface antigen expression.

Those cases with a high score may have progressed further along the premalignant pathway, and so tend to have a shorter survival. There is little correlation, however, between the score and other prognostic variables such as the bone marrow blast number and peripheral blood cytopenia, both of which tend to change adversely during the evolution of the disease. Though a weak correlation is seen with peripheral

blood haemoglobin concentration, this is difficult to interpret as some patients depend on transfusions. Surface antigen expression seems to be an independent prognostic variable.

The surface antigen score is closely related to subsequent leukaemic transformation. This is independent of the association with short survival, as many patients died in the absence of leukaemic transformation (table 2), often the case in this condition.³¹ The present association of high score and subsequent leukaemic transformation confirms the observations of Hokland *et al*,²⁶ who showed that cases with abnormal bone marrow mononuclear cell My7 expression had an increased tendency to evolve to RAEB and acute myeloid leukaemia.

CRA has been shown to induce changes in antigen expression^{9,32} and surface glycoproteins on HL60 cells.³³ Increases in antigens associated with maturing myeloid cells have been shown on acute non-lymphoid leukaemia blasts *in vitro*.³⁴ The present data show improvement in abnormal surface antigen expression on peripheral granulocytes and monocytes from some myelodysplastic patients treated with CRA. Serum concentration in patients treated with 20 mg CRA orally daily are about $1 \mu\text{mol/l}$.¹⁵ At this concentration both differentiation promoting and antiproliferative effects may be shown *in vitro* in normal CFU-GM^{10,35} and some leukaemic blasts,^{11,36} and an antiproliferative effect has been shown on CFU-GM in MDS.¹² The effect of CRA on surface antigen expression shown here may be mediated by inducing differentiation of normal progenitors, decreasing proliferation of abnormal progenitors, or a combination of these effects.

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