

Membrane Fc-IgG and C3b receptors on myeloid leukaemia cells: a comparison with cytoplasmic acid naphthyl acetate esterase cytochemistry

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SUMMARY Membrane receptors for IgG and C3b were examined on blast cells from 57 cases of acute myeloid leukaemia. These acute leukaemias were classified as myeloblastic, myelomonocytic or monocytic following morphological, cytochemical, and immunological investigations. The membrane receptors of leukaemic blast cells appear to be directly related to the degree of monocytic differentiation with the lowest receptor activities found in acute myeloblastic leukaemia. A comparison was also made between receptor and cytoplasmic acid naphthyl acetate esterase (ANAE) activities in 29 morphologically and immunologically-defined myelomonocytic and monocytic leukaemias. This study revealed that the receptor-positive "monocytic component" in a significant proportion of cases showed unexpectedly weak or negative ANAE reactions suggesting a more cautious approach to the interpretation of ANAE cytochemistry in acute leukaemias. The normal development of cytoplasmic ANAE and membrane receptors is also discussed and compared with their abnormal patterns of expression associated with leukaemic transformation.

The morphological classification of lymphoblastic leukaemias has been generally superseded by those based upon immunological characteristics.¹⁻³ The lack of specific myeloid markers has, however, meant that the classification of acute non-lymphoid leukaemias has been largely dependent on morphological and cytochemical investigations.

Previous reports indicate that leukaemic monocytes in acute myelomonocytic and monocytic leukaemias have membrane receptors for IgG or complement, or both^{4,5} while the percentages of receptor-positive normal granulocyte precursors are relatively low.^{6,7}

We have examined membrane IgG and C3b receptors in 57 cases of acute myeloid leukaemia which were classified as myeloblastic, myelomonocytic or monocytic following morphological, cytochemical and immunological investigations. In addition, the membrane receptor characteristics and cytoplasmic esterase cytochemistry of leukaemic monocytes were compared for their value in further subclassifying acute leukaemias of myeloid origin.

Material and methods

PATIENTS STUDIED

A total of 57 cases of acute myeloid leukaemia were examined and for this study were subdivided into myeloblastic (French-American-British groups M1 and M2; AML), myelomonocytic (FAB group M4; AMML) and monocytic leukaemias (FAB group M5; AMoL). These diagnostic categories were established by reference to morphological, cytochemical, and immunological criteria. Cytochemical investigations included peroxidase, chloroacetate esterase, acid naphthyl acetate esterase (ANAE) and butyrate esterases and isoelectric focusing of ANAE isoenzymes (manuscript in preparation). Monocytic involvement in the categories M4 and M5 was confirmed in 33 cases by the presence of monocyte-specific antigens detected by UCHM1, antilactoferrin, E11 and 45. This panel of antisera detects nearly all cases of myeloid leukaemia with monocytic involvement but does not stain leukaemic blasts from pure myeloblastic leukaemias (M1 and M2).⁸ The remaining cases (24) were categorised using monocyte-associated (although not completely specific) antisera, anti-

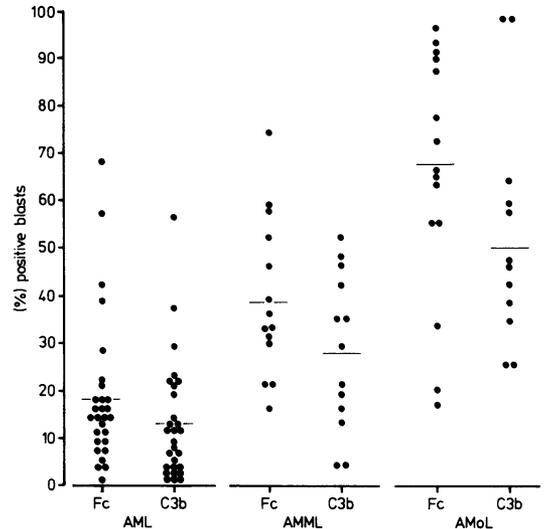
OKM1, anti-Mo2 and anti-monocyte (Clone 63D3, Bethesda Research Laboratories) in conjunction with cytochemistry.

RECEPTOR STUDIES

Rosette assays using IgG or human C3b-coated ox erythrocytes (oxE) for the demonstration of Fc-IgG and C3b receptors were performed as previously described.^{9,10} Receptor-positive leukaemic blasts, assessed following cyto-centrifugation of rosette preparations and Romanowsky staining, were expressed as the percentages of morphologically defined blasts binding in excess of three indicator particles.

ACID NAPHTHYL ACETATE ESTERASE (ANAE) CYTOCHEMISTRY

Blood smears or cyto-centrifuged leukaemic cell monolayers were stained for ANAE by the technique of Yam *et al*¹¹ using the substrate-diazonium salt combination of α -naphthyl acetate and hexazotised pararosaniline.



Fc-IgG and C3b receptor expression by blast cells in acute myeloblastic (AML), myelomonocytic (AMML) and monocytic (AMoL) leukaemias. Results are shown as percentages of rosette-forming blasts with mean values indicated.

Table 1 Comparison of membrane Fc-IgG and C3b receptors with cytoplasmic ANAE staining in acute myelomonocytic leukaemia (FAB group M4)

	Case											
	1	2	3	4	5	6	7	8	9	10	11	12
Cytoplasmic ANAE staining:												
Strength of reaction:*	+++	+	(+)	++	++	++	++	+++	+	+	++	(+)
% positive blasts:	42	5	5	49	5	24	35	55	30	60	80	5
Membrane receptors:												
Fc-IgG % rosette-forming blasts.	39	46	58	74	59	16	52	36	ND	21	46	43
C3b % rosette-forming blasts.	52	21	29	48	46	ND	42	35	16	13	24	56

All cases were classified by morphological and antigenic studies.

*All reactions are diffuse; (+) weak, + weak-moderate, ++ moderate, +++ strong.

ND = not done.

Table 2 Comparison of membrane Fc-IgG and C3b receptors with cytoplasmic ANAE staining in acute monocytic leukaemia (FAB group M5)

	Case																	
	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	
Cytoplasmic ANAE staining:																		
Strength of reaction:*	+++	++	+++	++	+++	+++	+++	+++	+++	+++	+++	+++	(+)	(+)	++	+++	(+)	+++
% positive blasts:	86	82	90	64	92	88	74	94	95	72	96	90	90	93	96	92	97	
Membrane receptors:																		
Fc-IgG % rosette-forming blasts.	87	77	17	66	65	91	33	63	72	60	20	96	93	94	55	89	91	
C3b % rosette-forming blasts.	25	47	42	46	59	52	4	54	64	45	ND	ND	ND	98	25	94	29	

All cases were classified by morphological and antigenic studies.

*All reactions are diffuse; (+) weak, + weak-moderate, ++ moderate, +++ strong.

ND = not done.

Results

FC-IGG AND C3b RECEPTOR EXPRESSION BY LEUKAEMIC BLASTS

The expression of Fc-IgG and C3b membrane receptors by blast cells in acute non-lymphoid leukaemias are shown in the Figure. The results show that the percentages of receptor-positive blasts are related to the degree of monocytic involvement in the leukaemic process. Thus, low numbers of receptor-positive blasts are seen in M1/M2 (Fc-IgG receptors, mean 18.4% SE 3.0; C3b receptors, mean 12.9% SE 2.4), high numbers are found in M5 (Fc, mean 67.6% SE 6.7; C3b, mean 52.3% SE 7.0) and intermediate numbers are found in M4 leukaemias (Fc, mean 39.2% SE 4.4; C3b, mean 28.0% SE 4.6). For comparison, examination of normal myeloblasts ($n = 283$) from 18 haematologically normal bone marrows revealed that a mean of 18% were Fc-IgG receptor positive.

COMPARISON OF MEMBRANE RECEPTOR AND CYTOPLASMIC ANAE ACTIVITIES OF LEUKAEMIC BLAST CELLS IN M4 (AMML) AND M5 (AMoL)

Twenty-nine cases of acute non-lymphoid leukaemia showing complete morphological and immunological diagnostic agreement were further examined for cytoplasmic ANAE and membrane receptors. The results of these comparative studies in M4 ($n = 12$) and M5 ($n = 17$) are shown in Tables 1 and 2 respectively. The ANAE activity of the "monocytic component" in M4 was often weak although some cases showed strong reacting cells. The percentages of receptor-positive blasts in M4 were generally in agreement with the percentages showing ANAE reactivity although two cases (10 and 11) had considerably lower numbers of receptor-positive cells than suggested by ANAE cytochemistry while four cases (2, 3, 5, and 12) showed a significant number of receptor-positive, ANAE-negative blasts. In M5 leukaemias, 11/17 cases showed high numbers of receptor-positive and strong ANAE-reacting blasts. Three cases (24, 25 and 28) exhibited high receptor activities with the vast majority of blast cells showing very weak diffuse ANAE reactions and three cases (15, 19, and 23) comprised blasts which were predominantly ANAE-positive and receptor-negative. For comparison, 24/28 cases of M1/M2 showed low numbers of receptor-positive blasts (<30%) and of the four cases with increased receptors, two had significant numbers of weak-diffuse ANAE positive blasts.

Discussion

This study has examined the expression of Fc-IgG

and C3b receptors by blast cells in a large number of acute non-lymphoid leukaemias which were categorised after extensive investigations. The results confirm previous observations^{4,5,12} with smaller groups of patients, that blast cells in leukaemias with monocytic involvement often show detectable membrane receptors whilst blast cells from "pure" myeloblastic leukaemias are usually receptor-negative. This study also reports that the numbers of receptor-positive M1/M2 blasts are similar to that seen with normal myeloblasts.

The main finding in this study, however, is the difference in the presence of receptors for the Fc of IgG and C3b and ANAE activities of blast cells in M4 and M5 leukaemias. Usually leukaemic myeloblasts, in common with normal myeloblasts, lack membrane receptors and show only weak, fine, granular or focal ANAE reactivity (personal observations). In contrast, "monoblasts" in typical cases of M5 show avid receptors and moderate to strong diffuse cytoplasmic ANAE reactions. In addition to these quantitative differences in ANAE cytochemistry, the ANAE isoenzyme patterns of M1/M2 and M4/M5 blasts, as assessed by isoelectric focusing, also show significant differences (manuscript in preparation). Of the 29 morphologically and immunologically defined M4 and M5 cases in the second part of this study, seven showed receptor activities on blast cells which lacked significant cytoplasmic ANAE reactivity whilst five cases showed a reverse pattern.

The expression of membrane receptors during granulocyte differentiation is well established^{6,7,10} while receptor expression by different stages of monocytes is less clear. There is increasing evidence, however, to suggest that the synthesis and development of membrane receptors is induced by extracellular proteins variously termed macrophage/granulocyte inducer (MGI)¹³ and Fc receptor inducer (FcRI).¹⁴ As cellular receptor synthesis may involve modulation of intracellular mechanisms by these extracellular factors, then the absence of receptors on cells showing antigenic and cytochemical characteristics of monocytes may indicate defects in the regulatory processes mediating receptor production.

Similarly, the synthesis of acid naphthyl acetate esterases, thought to be subject to differential gene activation in differentiating myeloid cells,^{15,16} may also be affected by leukaemogenesis and the absence of significant cytoplasmic ANAE activity in otherwise well defined receptor-positive monocytic cells supports this contention. Indeed, the transition of precursor cell ANAE isoenzyme synthesis to production of granulocyte or monocyte-specific esterases has been shown to be defective in a significant

proportion of "preleukaemic" (myelodysplastic) syndromes and non-malignant secondary dysplasias (megaloblastic anaemia).¹⁷ Furthermore, we have also examined a case of chronic myelomonocytic leukaemia (CMML) in which well differentiated peripheral blood monocytes, originally found to show strong ANAE reactivity, became ANAE negative over a six-month period even though the receptor and immunological characteristics of these monocytes were unchanged. Isoelectric focusing of the acid naphthyl acetate esterases from this case revealed that the loss of cytochemical ANAE activity was due to a marked reduction in the concentration of monocyte-associated isoenzymes.

In conclusion, this study has shown that receptor and ANAE characteristics of monocytes may be aberrant in acute leukaemia and that subclassification of acute myeloid leukaemia based upon a single investigation may be misleading.

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