Surface marker and other characteristics of Gaucher's cells

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SUMMARY A full surface marker study of the splenic storage cells in a case of Gaucher's disease largely substantiates the monocyte/histiocyte nature of Gaucher's cells. In addition, an apparent T-lymphocyte deficiency is demonstrated in the spleen and peripheral blood, and the possible significance of this finding is discussed.

Gaucher's cells have been extensively investigated morphologically and biochemically, and it is now thought that they represent histiocytic cells in which large amounts of glucocerebroside have accumulated because of a deficiency of β -glucosidase (Brady, 1972). The histiocytic nature of Gaucher's cells has been inferred from their phagocytic ability (Lee *et al.*, 1967), high lysosomal enzyme content (Rozenszajn and Efrati, 1961), and distinctively reticuloendothelial distribution. However, this view has not been universally accepted (Roos *et al.*, 1961; Fisher and Reidbord, 1962) and, in addition, immunological mechanisms have been invoked in the pathogenesis of the disease (Pennelli *et al.*, 1969). Immunological surface marker studies, which have proved of great value in the characterisation of the pathological cells of a wide variety of other haematological proliferations, should therefore contribute to further understanding of the nature of the pathogenomic cells of Gaucher's disease.

No previous surface-marker study of Gaucher's disease has been described, and in the present paper we report such a study in a case of the non-neuronopathic form of the disease.

Patient and methods

CASE HISTORY

This 8-year-old girl of Anglo-Saxon descent presented with recurrent epistaxes. Examination revealed massive splenomegaly and moderate hepatomegaly but no other abnormality. The serum acid phosphatase was greatly increased at 17.2 KA units, and skeletal

survey showed early, but definite, Ehrlenmeyer-flask deformity of the femora. There was a peripheral pancytopenia (Hb 10.7 g/dl, WBC $3.8 \times 10^9/l$, platelets $60 \times 10^9/l$) and differential white count showed 44% neutrophils, 49% lymphocytes, and 7% monocytes. A bone marrow aspirate was hypercellular and contained moderate numbers of typical Gaucher's cells; the cytology and cytochemistry of these are described in detail below. A diagnosis of non-neuropathic Gaucher's disease with hypersplenism was made.

The only other sib, a younger sister aged 5½ years, also has hepatosplenomegaly with peripheral pancytopenia and high serum acid phosphatase and almost certainly has Gaucher's disease. However, since she is asymptomatic a bone marrow aspirate has not been performed to confirm the diagnosis. There was no other family history of Gaucher's disease.

Because of the patient's thrombocytopenic bleeding attributable to hypersplenism, splenectomy was performed in December 1976 and this splenic material formed the basis of our study. Postoperatively, the patient has done well, and the pancytopenia has disappeared with cessation of the thrombocytopenic epistaxes.

CYTOLOGY AND CYTOCHEMISTRY

Bone marrow and splenic impression smears were examined by a variety of routine methods including polarised and ultraviolet microscopy, Romanowsky, PAS, acid and alkaline phosphatase, iron, peroxidase, Sudan black, α -naphthyl butyrate esterase with sodium fluoride inhibition, and naphthol AS-D chloroacetate esterase stains. Bone marrow and splenic material, fixed and processed in the manner

described below, were also examined by electron microscopy.

PREPARATION OF CELL SUSPENSIONS

Splenic cells

A single cell suspension was obtained by forcing several pieces from different areas of freshly removed spleen through a 120 gauge stainless-steel mesh into a Hepes-buffered Hanks balanced salt solution (HBSS). The cell suspension was kept on ice until its return to the laboratory where the cells were washed in HBSS, and mononuclear cells were separated by centrifugation over Ficoll-Hypaque gradients (Böyum, 1968). The cells were washed a further three times in HBSS before being resuspended in HBSS + 0.2% bovine serum albumin (BSA) to a final concentration of 2×10^6 cells/ml.

For comparison, suspensions from a 'normal' spleen (removed during gastrectomy for carcinoma of the stomach) and the spleen from a patient with hereditary spherocytosis (HS) were prepared in a similar way.

Peripheral blood cells

The patient's peripheral blood was examined on two occasions—initially, five days after splenectomy and, on a second occasion, 35 days post-splenectomy. On the latter occasion, the sister's blood was also examined. In each case, the blood was collected into heparin and the mononuclear cells were separated in the manner described above.

In both the splenic and the peripheral blood cell suspensions viability was assessed by the cells' ability to convert fluorescein diacetate to fluorescein (Celada and Rotman, 1967). Viability was greater than 95% in the peripheral blood, HS, and normal cell preparations. However, in the suspension from the Gaucher's spleen preparation the overall viability was 80%, while the Gaucher's cells, which constituted approximately 30% of this preparation, showed some 50% viability, dead Gaucher's cells accounting for the great majority of the non-viable cells.

ROSETTE TESTS

E-rosettes

Sheep erythrocyte rosette tests (E) were carried out using aminoethylisothiuronium bromide-treated erythrocytes according to the method of Kaplan and Clark (1974).

Fc-rosettes

The method of Hallberg *et al.* (1973), using ox RBCs sensitised with rabbit anti-ox RBC IgG antibody, was employed. Unsensitised washed ox RBCs were

used as an Fc control.

EA_{IgM}-rosettes

Ox RBCs coated with rabbit anti-ox RBC IgM antibody (EA_{IgM}) were used by a method previously described by us (Barker *et al.*, 1976; Burns *et al.*, 1977). This test also served as a control for the C3 rosettes.

C3-rosettes

(a) *C3bmo* and *C3dmo*¹ For C3bmo indicator cells, IgM-coated ox RBCs (0.5 ml) were pre-warmed to 37°C and an equal volume of whole AKR mouse serum (1/10 dilution) was added. These were mixed for 70 s at 37°C and 5 µl Antrypol^(R) (50 mg/ml), which stabilises C3b against the action of KAF, was added and thoroughly mixed for 2 min (Lachmann *et al.*, 1973).

As a source of C3dmo-coated cells, the reaction was allowed to proceed at 37°C without added Antrypol for 30 min during which time most C3b is converted to C3d by KAF.

(b) *C3bhu* and *C3dhu*² These two types of indicator RBC were prepared in an identical way except that human serum previously exposed to zymosan (R₃ reagent) was used as the source of complement (McConnell and Hurd, 1976).

All the C3 rosette tests were performed by the method of Ross *et al.* (1973).

Mouse (M-) rosettes

The method of Gupta *et al.* (1976), using fresh, washed, unsensitised CBA mouse erythrocytes was employed.

In all rosette tests, the indicator RBCs were washed three times and resuspended to 1% in HBSS + 0.2% BSA before testing, and percentages of rosetting cells were assessed using the fluorescein diacetate method of Ramasamy (1974), scoring only the viable fluorescing cells under combined UV and phase contrast microscopy.

SURFACE IMMUNOGLOBULIN (SMIg)

An indirect method was employed which uses polyvalent rabbit anti-human gammaglobulin raised in the laboratory followed by fluoresceinated goat anti-rabbit IgG (Gibco Bio-Cult Ltd, Paisley, Scotland).

¹C3bmo is the bound fragment of activated mouse complement component C3, and C3dmo is the residual fragment of the inactivation of C3b by conglutinin activating factor (KAF).

²C3bhu and C3dhu are the corresponding human complement components.

Fig. 1 EM of Gaucher spleen ($\times 8500$). A mature plasma cell (P) is seen to be intimately associated with two typical Gaucher's cells (G).

Fig. 2 *Fc-rosettes preparation of splenic Gaucher's cell suspension. The ability of the Gaucher cell to form Fc-rosettes is well shown at the level of both the light (inset) and electron microscope. In the electron micrograph ($\times 12\,000$) the Gaucher cell displays the imperfect fixation not infrequently observed in rosette preparations: this is presumably attributable to handling before fixation and was particularly marked in the case of the Gaucher cells.*

The cytocentrifuge preparation (inset) shows the strong Fc-rosette typical of the intact Gaucher cells. The associated plasmacytoid cell on the left is also clearly seen to be forming an Fc-rosette.

ELECTRON MICROSCOPY (EM)

Bulk preparations of rosettes were formed by allowing 1 ml of the splenic cell suspension at 2×10^6 /ml to react with 1 ml indicator erythrocytes. After centrifugation, the pellets were fixed in 3% glutaraldehyde and processed for EM by routine methods (Cawley and Hayhoe, 1973).

Results

CYTOLOGY AND CYTOCHEMISTRY

Both by light and electron microscopy, the large storage cells contained the striated inclusions which characterise Gaucher's disease (Figs 1 and 2). The cytochemistry was also typical of Gaucher's disease in showing strong PAS, acid phosphatase, and β -naphthyl butyrate esterase activity (Fig. 3a), which was partially inhibited by sodium fluoride.

The cells showed weaker alkaline phosphatase, iron, and naphthol AS-D chloroacetate esterase reactivity and were peroxidase and Sudan black negative.

Under polarised light the striated inclusions showed the form birefringence associated with some lipids (Bennett, 1950); in contrast, we have noted that the inclusions of sea-blue histiocytosis are not birefringent (R. J. Flemans; unpublished observation). Under UV illumination, the Gaucher's cells did not autofluoresce, again in contrast to both the storage cells of sea-blue histiocytosis and many bone marrow reticuloendothelial cells in a variety of non-storage conditions which fluoresce a strong pink (R. J. Flemans; unpublished observations).

ROSETTE TESTS

The results of the various splenic-cell rosette tests are summarised in Table 1. It is seen that a high

Fig. 3 Gaucher's cells. (a) α -naphthyl butyrate esterase preparation. The strong positivity of the Gaucher's cell is well shown. (b) E-rosette preparation; phase contrast, illumination. This positive rosette is typical in showing relatively few adherent sheep RBCs. (c) Fc-rosette formation; phase contrast illumination. This type of strong rosette with many adherent cells is typical. (d) Mouse erythrocyte-rosette formation; phase contrast illumination. The moderate number of positive, tended to be less strong than the Fc rosette.

percentage of Gaucher's cells formed rosettes with unsensitised sheep (E) RBCs (Fig. 3b), and with ox indicator cells coated with either the Fc of IgG (Figs 2 and 3c) or with human C3 (both C3b and C3d). A high percentage of the Gaucher's cells also formed rosettes with unsensitised mouse (M) erythrocytes (Fig. 3d), while a much lower percentage formed rosettes with ox indicator cells coated with mouse C3d. No rosette formation was seen with C3bmo-coated indicator cells. The Fc control (unsensitised ox RBCs) and the C3 control (EA_{IgM}) were also completely negative.

As is illustrated in Fig. 3 (b-d) the number of adherent indicator cells varied in the different rosette tests. Thus, in the Fc and C3hu (not illustrated) rosettes, the large number of adherent RBCs probably indicates high receptor density. In contrast, most Gaucher's cells which formed rosettes with sheep RBCs had only four to five adherent RBCs, although a number had more than 10 adherent cells.

The mouse rosettes were of intermediate appearance, and this may indicate intermediate receptor density. It should be stressed that only viable cells were scored in all these rosette tests.

The percentage total spleen cells forming E-rosettes in the Gaucher's preparation was remarkably low: indeed, none of the many small lymphocytes present was seen to form E-rosettes. This was in marked contrast to the findings in the two other spleens examined (Table 1).

The results of our studies in the T- and B-cell content of peripheral blood are summarised in Table 2. In both the patient and her sister, the percentage of peripheral blood T-cells appeared markedly reduced, whether estimated by E-rosette formation or by an esterase method recently described in this laboratory (Higgy *et al.*, 1977).

In both the whole spleen tissue and the splenic cell suspension, the Gaucher's cells were frequently found to be closely associated with one or more

Table 1 Rosetting properties of splenic cell suspensions

Rosette test	% Rosette formation by viable splenic leucocytes			
	Gaucher's disease		'Normal'	HS
	Total cells	Gaucher's cells		
E	5	54	51	45
Fc	71	59	34	35
EA ₁ IgM	0	0	5	4
C3mo	C3bmo	0	ND	ND
	C3dmo	32	8	1
C3hu	C3bhhu	68	ND	ND
	C3dhu	58	ND	ND
M	7	53	2	ND

ND = not done

Table 2 Peripheral blood rosetting data

Patient	% Rosette formation by mononuclear cells		Esterase staining	
	E	Fc	T-cell	B-cell
Propositus 5 days post-splenectomy	24	44	26	71
Propositus 35 days post-splenectomy	40	58	45	34
Sister with Gaucher's disease	53	45	54	35
Normal 7-year-old girl	71	24	ND	ND

ND = not done

plasmacytoid cells (Figs 1 and 2 inset). These associated plasmacytoid cells formed strong Fc (Fig. 2 inset), and mouse, but no C3 or E rosettes.

SURFACE IMMUNOGLOBULIN

Whereas some small lymphocytes in the splenic cell preparation were positive for SmIg, both the Gaucher's cells and the associated plasmacytoid cells were uniformly negative for SmIg.

Discussion

The morphology of Gaucher's cells is so distorted by the accumulated glucocerebroside that firm conclusions about the basic nature of the cell are not possible at the level of either the light or electron microscopes. In addition, unlike typical marrow histiocytes, the Gaucher's cells do not autofluoresce under UV light. However, our cytochemical studies, like those of others (Rozenszajn and Efrati, 1961), are consistent with the assumed monocytic origin of these cells; in this regard, the α -naphthyl butyrate esterase positivity, inhibited by sodium fluoride, is particularly suggestive of a monocytic origin.

Our demonstration of strong Fc-rosette formation by Gaucher's cells, in the absence of SmIg, is also

in keeping with a monocyte/histiocyte nature. The presence of a high density of receptor for human C3 (both C3b and C3d) is again in accord with a monocytic/histiocytic origin of the Gaucher cell (Ross and Polley, 1975). The failure to form C3bmo rosettes, and only a low percentage of rosette formation with C3dmo, is difficult to interpret but may reflect either a lower density of C3 products on those indicator cells or the ability of the Gaucher's cells to distinguish between human and mouse C3. The ability of Gaucher's cells to form rosettes with sheep erythrocytes is also difficult to interpret. Normally, E-rosettes formed by T-lymphocytes of less than 10 μ diameter consist of a tight morula made up of several layers of indicator cells. In contrast, the much larger Gaucher's cells (> 40 μ diameter) usually displayed only relatively few adherent cells sparsely distributed over the cell surface. Although it is possible that Gaucher's cells possess a receptor similar to that of T-lymphocytes, in view of the other characteristics of the cells, it is difficult to imagine that the formation of rosettes with sheep RBCs is a true expression of a T-lymphocyte phenotype. Histiocytes are known to be capable of recognising and removing effete erythrocytes, and the rosettes seen with sheep RBCs may be related to this process. Some support is lent to this interpretation by our observation of weak E-rosette formation by the storage cells of a single case of sea-blue histiocytosis (Burns, unpublished observations). Because the mouse rosette test is generally considered to identify specifically a subpopulation of B-lymphocytes (Forbes and Zalewski, 1976) and the leukaemic cells in CLL (Stathopoulos and Elliott, 1974), the reactivity of the Gaucher's cell may also be attributed to the adherence of effete RBCs. However, in our hands, some normal monocytes and neutrophils form M-rosettes, and thus the ability of Gaucher's cells to form these rosettes may be fully compatible with a monocyte-histiocyte lineage.

In view of the immunological abnormalities that may be associated with Gaucher's disease (Pratt *et al.*, 1968), the severe deficiency of splenic T-cells demonstrated by the strikingly low percentage of E-rosette formation by the numerous lymphocytes present in the patient's spleen is of interest. This deficiency is clearly not attributable to sampling error since our splenic suspension was prepared from several pieces of tissue from widely different areas of the spleen. This unexpected finding prompted us to examine the T-cell content of the patient's peripheral blood. Interestingly, both the patient and her sister displayed a definite reduction in the percentage of circulating T-cells. These results cannot be simply attributed to a defect in our

E-rosette technique because it yielded an entirely normal result in a control child and because similar results were obtained by a completely different method employing esterase cytochemistry (Higgy *et al.*, 1977). Furthermore, the detection of T-lymphopenia in the sister argues against the finding in this patient being simply a response to the operation and/or its attendant anaesthetic. The possibility that the T-lymphopenia represents a manifestation of hypersplenism or an unrelated chance association cannot, however, be excluded.

The association between plasma cells and both normal reticuloendothelial cells and Gaucher's cells is well recognised, and the latter observation is confirmed in the present study. However, since the associated cells closely resembled mature plasma cells morphologically, and lacked SmIg, the presence of an Fc receptor on their surface is an unusual finding since plasma cells usually lack the Fc receptor (McConnell, 1975). We are unable to offer any definitive explanation for this finding. However, it has been suggested that plasma cells actively secreting immunoglobulin lack the Fc receptor while inactive plasma cells possess this marker (Ramasamy *et al.*, 1974); the Gaucher-associated plasma cells may therefore be inactive cells.

To conclude, the present marker study largely substantiates the assumed relationship between Gaucher's cells and cells of the monocyte/histiocyte series. However, some possible differences between Gaucher's and monocytoid cells were observed, but their significance awaits further investigations of both more cases of Gaucher's disease and the surface marker properties of the reticuloendothelial cells of normal spleen, for which no data are currently available. Moreover, the demonstration of an apparent T-cell deficiency in the present patient's spleen and peripheral blood suggests that examination of T-cell function in further cases of Gaucher's disease should be undertaken.

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