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Expression of MHC Class I determinants on erythrocytes of SLE patients

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SUMMARY

Strong expression of MHC Class I determinants had been observed on the erythrocytes of three genetically C4 deficient patients who all had SLE. In a study of 35 other SLE patients who were not C4 deficient, 30 showed a marked increase in the expression of MHC Class I on their erythrocytes. There was a correlation between the expression of erythrocyte Class I and disease activity. The polymorphic HLA determinants were detected by haemagglutination with human cytotoxic antisera from untransfused pregnant women. A shared monomorphic epitope of HLA-A, -B and -C, and β_2-microglobulin were detected by haemagglutination with monoclonal antibodies. A monoclonal antibody for a monomorphic epitope on MHC Class II α and β chains did not react. Erythrocytes from a group of RA patients and a group of normal controls had moderate and low expression respectively. We suggest that MHC Class I may be induced on erythrocytes maturing in a milieu containing mediators derived from activated cells of the immune system. Aberrant tissue expression of MHC antigens may be more widespread than has been previously recognized in diseases mediated by immune mechanisms.

Keywords HLA Class I erythrocytes SLE and RA patients

INTRODUCTION

Mature circulating human erythrocytes do not normally express easily detectable HLA Class I determinants though they can be demonstrated on reticulocytes and even persist for a short period after the nucleus has disappeared (Brown *et al.*, 1979). Platelets, the other non-nucleated peripheral blood cells, express HLA strongly but experimental data had suggested that this was acquired from plasma (Lalezari & Driscoll, 1982). However, Santoso *et al.* (1986), who investigated adsorption of HLA by platelets *in vitro* and *in vivo*, concluded that the major portion of platelet HLA is an integral part of the cell membrane.

In 1963, a group of human erythrocyte allo-antibodies was first described (Buchanan & Afaganis, 1963; Chown, Lewis & Kaita, 1963) but later collectively termed anti-Bg (Seaman *et al.*, 1967) and shown to detect determinants associated with HLA (Morton, Pickles & Sutton, 1969; Morton *et al.*, 1971). Studies with the AutoAnalyser increased the sensitivity of agglutination and many specificities were found that could be correlated with cytotoxic antibody specificities (Nordhagen, 1975; 1977). Some HLA determinants were generally expressed more strongly, such as B7 (Bg^a), and haemagglutination by anti-Bg^a in capillary tubes detected the antigen in 93% of HLA

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B7 individuals (Crawford & Schroeder, 1980). Monoclonal antibodies for the monomorphic epitope shared by HLA-A, -B and -C products and for β_2-microglobulin in radio-immunoassay and flow cytometry experiments detected HLA expression on the erythrocytes of 52% of donors and confirmed the strong correlation of detectable HLA with the presence of HLA-B7 (Rivera & Scornik, 1986). From these data, it appears that the HLA expressed on erythrocytes is similar to that on lymphocytes but at a much lower level. Exceptionally strong HLA reactivity apparently persisted in two normal donors (Nordhagen, 1979) and transiently increased in patients following an EB virus infection (Morton, Pickles & Harley, 1977).

The difficulty encountered in typing the erythrocytes of a C4 deficient patient for the lack of C4 antigenic determinants Rodgers (Rg) and Chido (Ch) (Giles & Swanson, 1984) was related to the presence of cytotoxic antibodies in the anti-Rg and anti-Ch reagents. Testing the cells of this C4 deficient patient and two others with a battery of selected human cytotoxic antisera (Giles *et al.*, unpublished) confirmed the presence of strong HLA reactivity. The majority of C4 deficient patients suffer from systemic lupus erythematosus (SLE) (Hauptmann *et al.*, 1986) so it was important to establish whether the aberrant HLA expression on erythrocytes correlated with C4 deficiency or SLE. By means of haemagglutination, a study with human polyclonal and mouse monoclonal antisera has demonstrated that most SLE patients have elevated HLA expression when compared with rheumatoid arthritis (RA) patients and normal donors.

MATERIALS AND METHODS

Patients. Fresh red cell samples (EDTA) were obtained at Hammersmith Hospital. Thirty-five patients classified as having systemic lupus erythematosus according to the 1982 revised ARA criteria (Tan *et al.*, 1982) and family members of eight of them were studied. The level of disease activity at the time of bleeding was determined using the LACC score method (Urowitz *et al.*, 1984). Patients were categorized as having active (LACC score $\geqslant 2$), moderately active (LACC score 1) or inactive disease (LACC score 0). A control group of 26 rheumatoid arthritis patients and 11 hospital staff members were also bled.

Fresh red cells from blood donors were kindly provided by the Regional Blood Transfusion Centres in Oxford and South London. Some were bled into EDTA and others in CPD. Cells known to have Bg antigens (Bg^a, Bg^b, Bg^c) were supplied by Mr P. Bowell (Oxford).

Antisera. Seven human lymphocytotoxic antisera (group A) with broad specificity were selected for use as a small screening panel. The antisera came from women stimulated by pregnancy alone and had a reaction frequency of more than 80% with a random lymphocyte panel; there were no detectable erythrocyte antibodies when these antisera were tested against a 10-cell donor panel issued by the Oxford Regional Transfusion Centre to their hospitals. Three mouse monoclonal antibodies, W6/32 (Barnstaple *et al.*, 1978), BBM 1 (Brodsky, Bodmer & Parham, 1979) and CA2.06 (Charron & McDevitt, 1979) were kindly provided by Dr J. Bodmer (ICRF) in the form of supernatants. Antiglobulin reagents for direct tests were gifts (from Mr J. Booth, Mrs B. Gardner, Professor G. Hauptmann, Mr P. Howell, Professor P. Mollison, Professor P. Lachmann and Dr D. Voak).

Direct antiglobulin tests. These were performed on a tile with a battery of optimally diluted antisera; anti-IgG (two reagents), anti-C4 (C4d), anti-C4c, anti-C4d + C3d, anti-C3 (C3d) and a polyspecific reagent standardized for use in blood banking.

Indirect agglutination tests. These were performed in capillary tubes (Chown, 1944). Human cytotoxic antisera and mouse monoclonal reagents were used with anti-human IgG diluted 1 in 50 and anti-mouse IgG diluted 1 in 32 respectively. To enhance agglutination by the mouse antisera, red cells were pretreated with a 1% papain solution (Giles & Swanson, 1984).

Table 1. Some typical haemagglutination results with selected red cells

RBC samples	Antisera: Human cytotoxic reagents I	II	III	IV	V	VI	VII	Inert: AB serum	Mouse monoclonals: W6/32	BBM1	CA2.06
SLE patients											
F.F.	4	4	4	4	4	4	5	–	4	4	–
J.N.	2	1	3	2	1	3	4	–	2	2	–
I.S.	–	–	–	–	–	–	–	–	2	2	–
G.R.	–	–	–	–	–	–	–	–	–	–	–
C4 def. patient, M.B.	4	4	3	3	3	4	4	–	4	4	–
Normal control											
2406 Bg (a+c)	–	1	3	–	1	NT	3	–	2	2	–
C.G. Bg (–)	–	–	–	–	–	–	–	–	–	–	–

1–4, score of positive reactions; –, negative reaction.
NT, not tested.

RESULTS

Direct antiglobulin testing

Patients. All the erythrocytes were tested for the presence of IgG, C3 and C4 on their surfaces. Cells coated with easily detectable IgG could not be screened with human IgG cytotoxic antisera. It was also of interest to assess whether the C4, and to a less extent C3, bound to patients' erythrocytes might correlate with the abnormal expression of HLA Class I. Eight of the 35 SLE patients had detectable IgG but only four of the reactions were sufficiently strong to interfere with indirect anti-IgG tests. Six SLE patients had detectable C3. None of the 26 RA patients reacted with anti-IgG or anti-C3. C4 was detected on all but four of the SLE patients' erythrocytes, 22 were of moderate strength and the rest (nine) were weak. In contrast, only seven RA patients had a moderate reaction and the remainder were weak (15) or negative (four). The samples did not react with anti-C4c, so it was deduced that C4d was the predominant C4 component detected.

Normal controls. None of the 44 fresh control samples reacted with anti-IgG or anti-C3 and only one reacted weakly with anti-C4.

The sensitivity of the tile test is not great but nevertheless was sufficient to demonstrate differences in uptake of IgG, C3 and C4 between the two disease groups and normal controls.

Tests for HLA (Class I) determinants

All of the group O and A erythrocytes were tested against human polyspecific (Class I) cytotoxic antisera. The seven antisera were selected for use initially with the group A cells of the C4 deficient patient (Giles & Swanson, 1984) and should have been suitable for the majority of patient samples. However there were eight group B SLE patients, one B and two AB RA patients and one B control that had to be excluded from these tests. All erythrocytes, regardless of the ABO group, were tested with the mouse monoclonal antisera. The human antisera react with determinants on the two variable domains of the Class I polypeptide whereas the monoclonal antisera react with the constant region of Class I and Class II and with the invariable β_2-microglobulin.

Table 1 shows patterns of reactivity obtained with some SLE patients, the C4 deficient patient (M.B.) reported by Giles & Swanson (1984) and normal controls. The human antisera gave uniformly positive reactions when the expression was strong (patient F.F.) but possible differences in the specificities were demonstrated by samples with less strong expression of HLA (patient J.N.) and it is presumed these would relate to the HLA type of the individual. The erythrocytes of one

Table 2. Human cytotoxic antisera reactivity with red cells of O and A groups by the IgG antiglobulin technique expressed as scores

	No. of samples	>20 (%)	>10 (%)	>2 (%)	0 (%)
SLE patients*	23	14 (61)	5 (22)	2 (8·5)	2 (8·5)
RA patients	23	3 (13)	9 (39)	6 (26)	5 (22)
Normals†	45	2 (4·5)	2 (4·5)	17 (38)	24 (53)

* Only patients with a negative direct IgG antiglobulin reaction were tested.

† Bg status unknown.

patient (I.S.) did not react with the human antisera but nevertheless did react with the monoclonal antisera W6/32 and BBM 1. Her HLA type was not unusual (A 2, 28; B 14, 15; Cw3) so this single anomalous result cannot be attributed to her HLA type and the antibody specificities in the human antisera.

The two Class I monoclonal antibodies W6/32 and BBM 1 were used at dilutions of 1 in 8 and 1 in 16 and gave equal strength reactions in all but the very weakest expression seen with some of the erythrocytes from RA patients. CA2.06 monoclonal was used as a negative control reagent. As no cells, except those with strong IgG coats, reacted with CA2.06, it has been impossible to claim that Class II determinants are not expressed on erythrocytes but it seems very probable. The papain-treated IgG coated cells were shown to react non-specifically with culture medium, CA2.06 and with a monoclonal reagent of an irrelevant specificity, anti-C4c. By using untreated erythrocytes instead of papain-treated RBC, the 'false positive' reaction was removed or reduced to a minimum.

Human cytotoxic antisera

The results are shown in Table 2. As human cytotoxic antisera are multispecific and detect different determinants of HLA A and B (Nordhagen, 1975; 1977), the strength of reactivity has been expressed as the score of the seven reagents. For example from Table 1, F.F. scores 29 and J.N. scores 16. None of the normal samples were known to be Bg-positive, though clearly the two with scores of more than 20 should be classified as such. One with a score of more than 10 was later confirmed as Bg (a+). Most SLE patients scored more than 20 and many more than 10, the majority of RA patients scored less than 20 and the normals less than 10. Samples of erythrocytes from seventeen Bg-positive donors were not all freshly taken and therefore excluded from Table 2. None had scores of 20, three had scores of more than 10 (18%), ten more than 2 (58%) and four were non-reactive (24%). These results demonstrate greater HLA reactivity than the normals but less than both patient groups.

Monoclonal antisera for Class I, and β_2-microglobulin

The results given in Table 3 are based on the strength of reactivity with W6/32 (Class I) and BBM 1 (β_2-m) which were used at dilutions that gave comparable results. The expression of monomorphic Class I and β_2-microglobulin show a similar distribution to that with the human antisera. SLE patients have stronger expression than RA patients and normal individuals have the least, but the percentages vary a little from those in Table 2 with the human antisera.

Family studies

Thirty-five family members of eight SLE patients have been studied with a view to assessing whether the strong expression of MHC Class I on erythrocytes was inherited with a particular haplotype. Unfortunately there were group B members in four families so only the results with the monoclonal antisera could be used. Four patients had HLA identical siblings without SLE whose cells did not react. Two HLA non-identical sibs both had SLE and HLA expressed on their cells. Clearly HLA

Table 3. Reactivity of monoclonal antisera for monomorphic Class I (W6/32) β_2-microglobulin (BBM 1) determinants

	No. of samples	Reaction strength			
		5:4 (%)	3:2 (%)	1 or < 1 (%)	0 (%)
SLE patients	34	18 (53)	12 (35)	2 (6)	2 (6)
RA patients	26	1 (4)	5 (19)	8 (31)	12 (46)
Normals	21	0 (0)	2 (10)	0 (0)	19 (90)

expression on erythrocytes is not controlled by the extended MHC haplotype. In three families, a single parent of the SLE patient expressed HLA strongly and in another three in which only one parent was available for testing, HLA was not enhanced. It is not yet possible to decide whether enhanced HLA expression is under genetic control, but this will continue to be examined.

Group O and A family members were tested with the cytotoxic antisera. Cells from one of the parents that reacted with the monoclonal antisera gave a score of more than 20. In four families, erythrocytes of eight direct relatives (parents, sibs or children) and one husband reacted quite strongly with human reagents but did not react with the monoclonal reagents. Further studies may elucidate this apparently anomalous reactivity.

Expression of HLA on the erythrocytes of SLE patients in relation to their clinical condition and other serological abnormalities

Twenty-five patients showed strong expression (reaction strength 5–3) and eight weak expression (reaction strength < 3) of Class I antigens on their erythrocytes as judged by haemagglutination with monoclonal antisera. One patient was tested only with the human cytotoxic antisera and is not included in this count. Clinical information was available on 33 out of 35 subjects at the time of bleeding. Twenty out of 25 patients with strong HLA expression had moderately active or active disease compared with only three out of eight patients with weak HLA expression ($\chi^2_1 = 3{\cdot}37$, $0{\cdot}1 > P > 0{\cdot}05$). Although C4 was detected on most of the erythrocytes from SLE patients, there was no correlation of the C4 levels in serum with the expression of HLA Class I.

DISCUSSION

We describe here a new example of anomalous tissue expression of HLA antigens in disease. Strong expression of HLA Class I antigens has been found on the erythrocytes from patients with SLE and weaker but still abnormal expression in patients with RA.

Our data show that many individuals, including normals, have some HLA Class I expressed on erythrocytes. Haemagglutination studies suggest that there may be quantitative variation from those whose erythrocytes fail to react to those with greatly enhanced reactivity. Further studies are in progress to enumerate directly the Class I antigens on erythrocytes and to assess whether the expression varies with clinical remissions and crises in individual patients.

It has not been clearly established whether Class I antigen expression on erythrocytes and platelets is due to their presence as intrinsic membrane antigens or adsorption from plasma. The main evidence in favour of adsorption comes from studies showing that platelets may acquire HLA specificities when incubated in plasma (Lalezari & Driscoll, 1982). In further support of this concept, there have been reports that showed with chloroquine it was possible to strip HLA specifically from platelets (Blumberg *et al.*, 1984), neutrophils (Minchinton & Waters, 1984) and red cells (Swanson & Sastamoinen, 1985). Chloroquine cleaves or inhibits antigen-antibody binding (Mantel & Holtz, 1976); it would not however be expected to cleave a polypeptide chain. In favour of the idea that expression on erythrocytes is secondary to its presence as an integral membrane

protein are the following findings. Nordhagen (1978) was unable to demonstrate HLA adsorption by red cells and considered there was no evidence to show that HLA was acquired from plasma. The amount of HLA in the plasma of individuals with enhanced expression on erythrocytes was not markedly raised (Nordhagen, 1979; Morton *et al.*, 1977). The Class I polypeptide structure has been well characterized (Owen & Crumpton, 1980) with four domains external to the cell membrane α 1, α 2, α 3 and the closely associated β_2-microglobulin. The α 1 and α 2 domains carry the polymorphic epitopes detected by human cytotoxic antisera and α 3 carries the monomorphic epitope detected by the monoclonal antibody W6/32 (Barnstaple *et al.*, 1978). The β_2-microglobulin is also invariable. Until recently, β_2-microglobulin had not been detected by serologists studying HLA on erythrocytes (Nordhagen, 1979). Mouse erythrocytes express Class I MHC (H-2) but they are able to absorb much less anti-H-2 than lymphocytes; nevertheless a recent study showed that the H-2 antigens isolated from erythrocytes and lymphocytes were structurally very similar (Spack & Edidin, 1986). Rivera & Scornik (1986) detected the W6/32 α 3 epitope and β_2-microglobulin by radio-immunoassay and flow cytometry and they suggested that human erythrocytes have the complete extracellular structures and therefore possibly also the transmembrane and intracellular structures. Our report is yet another demonstration of both variable and constant regions of the Class I polypeptide on erythrocytes and has been made possible by the marked enhancement on the erythrocytes of SLE and to a lesser extent RA patients.

We found a weak correlation between SLE disease activity and enhanced MHC Class I expression on erythrocytes, suggesting that the effect may be secondary to disease activity. A possible explanation is that erythrocyte precursors are stimulated at the nucleated stage of development to produce more MHC Class I polypeptides which persist for the life span of that cell in circulation. Anderson & Berkwitz (1985) demonstrated that gamma-interferon enhances the expression of MHC Class I in the human choriocarcinoma cell line BeWo that is weakly HLA-reactive. Reticulocytes are known to express HLA weakly (Brown *et al.*, 1979) so a parallel situation may exist. Gamma-interferon has an immuno-regulatory role and is likely to be found in patients as a result of auto-immune response triggered by T cell activation, IL-2 secretion and B cell activation. Whether the presence of Class I MHC on erythrocyte surfaces has any functional consequences for the patient is uncertain.

These studies indicate that aberrant tissue expression of MHC antigens may be widespread in patients with diseases putatively mediated by immunological mechanisms.

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