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The E2 antigen, a 32 kd glycoprotein involved in T-cell adhesion processes, is the MIC2 gene product

Catherine Gelin, Francoise Aubrit, Armelle Phalipon[1], Brigitte Raynal, Stewart Cole[2], Michel Kaczorek[3] and Alain Bernard

Laboratoire d'Immunologie des Tumeurs de l'Enfant, Pavillon de Recherche, Institut Gustave Roussy, rue Camille Desmouslins, 94800 Villejuif, [1]Laboratoire des Entérobactéries, Institut Pasteur, 28 rue du Docteur Roux, 75015 Paris and [2]Laboratoire de Biochimie des Régulations Cellulaires, Institut Pasteur, 28 rue du Docteur Roux, 75015 Paris, France
[3]Present address: Pasteur Vaccin, BP 101,27001 Val de Reuil, Cedex, France

E2 is a 32 kd human T-cell surface glycoprotein involved in spontaneous rosette formation with erythrocytes. A 1.11 kb cDNA was isolated from a λgt11 expression library by screening with monoclonal antibodies directed against E2. The primary structure of E2, deduced from the nucleotide sequence of its gene, comprises 185 amino acids and is devoid of *N*-linked glycosylation sites. The E2 protein is rich in proline residues and displays an organization typical of an integral membrane protein. Northern blotting showed a good correlation between mRNA abundance, E2 surface density and the level of T cell differentiation. In fact, nucleotide sequencing revealed that E2 is the MIC2 gene product, previously identified with the 12E7 Mab. Xg(a$^-$) female individuals have no E2 molecule on the surface of their red cells, in contrast with Xg(a$^+$) individuals, but have the molecule in their cytoplasm, in the form of the 28 kd precursor. These findings show that the E2 antigen, a cell surface molecule involved in T cell adhesion processes, is the product of the MIC2 gene, the only pseudoautosomal gene to be described in man.

Key words: MIC2 gene/T-cell rosettes/T-cell surface antigen/Xg blood group

Introduction

Spontaneous rosette formation between human T cells and erythrocytes from various species (Fröland, 1972; Jondal *et al.*, 1972; Amiot *et al.*, 1984) has been a very useful phenomenon to identify, enumerate and separate human T lymphocytes. It has allowed significant progress in our knowledge of human T cell physiology, and has been used in therapy (Reisner *et al.*, 1983). The significance of this apparently artificial phenomenon has been clarified by the identification of the CD2 (T, gp50) molecule, a T cell surface antigen responsible for this interaction (Kamoun *et al.*, 1981; Bernard *et al.*, 1982) involved both in T cell activation (Meuer *et al.*, 1984; Reed *et al.*, 1985; Brottier *et al.*, 1985; Schmidt *et al.*, 1985; Huet *et al.*, 1986) and T-cell adhesion processes (Shaw *et al.*, 1986; Vollger *et al.*, 1987). In fact, a complex pattern of signalling can be delivered to the T cell via CD2, probably determined by the conformational changes imposed on this molecule (Bernard *et al.*, 1986). A ligand for CD2 has been identified on sheep erythrocyte surface as being a 42 kd protein, termed T11TS (Hünig *et al.*, 1987). The human cell surface equivalent for T11TS is the LFA3 molecule, a glycoprotein of mol. wt 55–70 kd, broadly distributed on non-haematopoietic and haematopoietic cells (Selvaraj *et al.*, 1987). However the complex role of the CD2 molecule in different aspects of human T cell physiology suggested that it might interact with more than a single ligand. Thus, we have identified two other molecules from sheep erythrocyte surface, involved in rosette formation (Bernard *et al.*, 1987), and termed S14 and S110-220, according to their mol. wts. Recently, we have described the existence of the putative human counterpart of the 14 kd protein, termed H19, expression of which is not limited to the erythrocyte surface but also found on many nucleated cells including T cells and monocytes, and which is involved in T cell activation (Groux *et al.*, 1989).

Just as three different erythrocyte surface molecules are required in spontaneous rosette formation, we have demonstrated the presence of a 32 kd molecule on the T-lymphocyte surface, different from CD2, which is also involved in this interaction (Bernard *et al.*, 1988). This molecule, termed E2, has been identified by using four monoclonal antibodies (mAb) which block rosette formation and do not react with CD2. Recent biochemical studies (Aubrit *et al.*, 1989) have shown that this 32 kd protein is highly glycosylated, the sugar residues accounting for 14 kd in the mol. wt. This study has revealed no evidence for *N*-linked polysaccharide chains and all the sugar residues appear to be *O*-linked. A 28 kd precursor species has been identified that probably corresponds to the unsialylated, fully glycosylated molecule. The 15 N-terminal amino acid residues have been determined from the E2 purified molecule but showed no homology to any known proteins. The surface density of E2 changes with the differentiation level of T cells: present at high density on thymocytes and less well expressed on peripheral blood T lymphocytes. Unlike CD2, its density is not increased during activation.

Cloning the genes encoding these different molecules might be very helpful to understanding the complex interactions involved in the rosette formation phenomenon. In this paper, we describe the isolation and characterization of E2 cDNA and its application to studying the structure of E2 and its function in T cell adhesion.

Results

Isolation of E2 cDNA clones

A cDNA clone encoding the E2 polypeptide was identified by screening a λgt11 expression library constructed from the poly (A$^+$) mRNA of human thymocytes, with a mixture of two different E2 mAb (0662 and L129) that we have

Fig. 1. Nucleotide sequence of the E2 cDNA and deduced primary structure of the protein. The signal peptide, the N-terminal sequence determined by gas phase sequencing of the E2 purified molecule and the transmembrane region are overlined. Numbers in the right margin show nucleotide positions, whereas numbers above amino acid sequence indicate residue positions. The potential polyadenylation signal AATTAAAA is underlined.

previously obtained. Five positive clones were obtained by screening 2.5×10^5 transformants. Specificity was demonstrated by the failure of their β-galactosidase fusion proteins to react with a negative control antibody or with monoclonal antibodies of different specificity. Two clones, BC1 and BC2, yielded a cDNA insert of 1.1 kb after digestion with *Eco*RI. The cDNA of BC1 was subcloned into plasmid pUC8 and the nucleotide sequence of the insert was determined after further subcloning into M13mp18.

Nucleotide and amino acid sequences

The complete coding sequence of the E2 gene was obtained by sequencing both strands of the BC1 clone. The 1110 bp sequence contains a short 5′ untranslated region and a 0.45 kb 3′ untranslated region with a consensus polyadenylation signal at position 1083. The open reading frame begins with the first ATG at position 122, ends with a TAG termination codon at position 679, and encodes a predicted polypeptide of 185 amino acids (Figure 1). The presence of a cytosine 1 bp and an adenine 3 bp, 5′ of the AUG, is consistent with this being close to an optimal higher eukaryotic translational start site (Kozak, 1986).

The first 21 residues are characteristic of a signal peptide and this is followed by a sequence that matches 12 of the 14 N-terminal amino acids previously determined for the purified E2 molecule (X-X-L-F-D-L-S-D-A-L-P-P-N-E-N-X-K). Two discrepancies can be noted (residue 3 L instead of G; residue 12 D instead of P), but overall the N-terminal amino acid sequence deduced from the cDNA sequence is in excellent agreement with that determined for the purified molecule.

After removal of the signal peptide, the native E2 protein comprises 163 amino acids. The predicted N-terminal domain, consisting of 100 amino acids, is followed by a hydrophobic region of 25 residues, characteristic of a transmembrane domain (Eisenberg *et al.*, 1984). This region immediately precedes a predominantly hydrophilic C-terminal region of 38 amino acids.

The calculated mol. wt for the native E2 is 16 710 although when glycosylated it migrates with an apparent mol. wt of 32 000 on SDS−polyacrylamide gels. A substantial amount of glycosylation must occur to account for this size difference and this is likely to be *O*-linked as there are no candidate sites for *N*-linked glycosylation. This is in agreement with results obtained by Aubrit *et al.* (1989) who have found E2 to be resistant to endoglycosidase F, and have shown that after *O*-glycanase treatment the mol. wt is reduced to 18 000. Moreover, the presence of 19 basic and 29 acidic amino acids in the predicted processed protein sequence agrees with the determined acidic pl (4.5) of E2. Thus the biochemical

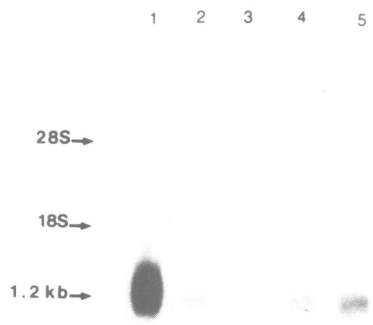

Fig. 2. RNA Blot analysis of different cell lines. Northern blots were hybridized with the ^{32}P-labelled E2 cDNA. Poly (A^+) mRNAs (20 μg) from HDMAR (**lane 1**), U937 (**lane 2**), Daudi (**lane 3**), peripheral blood lymphocytes (**lane 4**) and thymocytes (**lane 5**).

properties of the predicted protein encoded by this cDNA are consistent with those described for the mature E2 glycoprotein. It must be noted that the E2 amino acid sequence revealed no cysteine residues and this means that the E2 mature protein would have no disulphide bridges.

mRNA expression

Equal amounts of poly (A^+) mRNA prepared from several cell types were electrophoresed in denaturing agarose gels and transferred to nylon membranes. Using the cDNA clone BC1 as a probe, the hybridization experiments revealed the presence of 1.2 kb mRNA transcripts both in thymocytes and HDMAR cell (Figure 2). Lesser amounts were found in RNA extracted from peripheral blood cells and the cell line U937 whereas no hybridization was observed with mRNA from the B cell line Daudi. The hybridization pattern is in agreement with the pattern of expression of the E2 protein which is (i) undetectable on the B cell line Daudi, (ii) present at high density on thymocytes and on HDMAR cell line, and (iii) less abundant on peripheral blood lymphocytes and the monocytic cell line U937 (Bernard *et al.* 1988).

Homology of E2 to other proteins

Comparison with nucleotide sequences contained in the EMBL bank (Lipman and Pearson, 1985) revealed that the E2 cDNA was identical with the MIC2 cDNA as described by Banting *et al.* (1988). The product of the MIC2 gene was first identified by using the 12E7 mAb (Levy *et al.*, 1979); this antigen has been described as a 30 kd protein present on almost all types of human cells and has been shown to be independently encoded by a pair of related genes present on the human X and Y chromosomes. From results obtained by Banting *et al.* (1988) we can conclude that the N-terminal

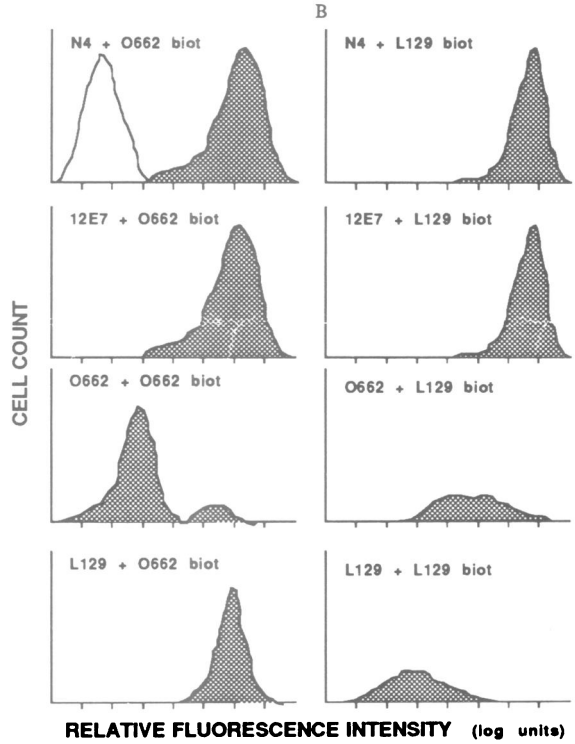

Fig. 3. The 12E7 mAb recognize the E2 molecule (**A**) and define a third epitope on the molecule (**B**). (**A**) The E2 molecule was purified from membranes of thymocytes as described (Aubrit *et al.*, 1989). The purified E2 molecule was then subjected to electrophoresis and probed in Western blot analysis with 12E7 mAb (**lane 1**), 0662 mAb (**lane 2**) or with an unrelated mAb (P296) (**lane 3**). (**B**) Fluorescence histograms of competitive binding experiments between 12E7, 0662 and L129 mAb. Target cells were thymocytes. The second antibody was coupled to biotin, as indicated by 'biot' and revealed by fluoresceinated avidin. The negative control (N4) is represented as clear histogram.

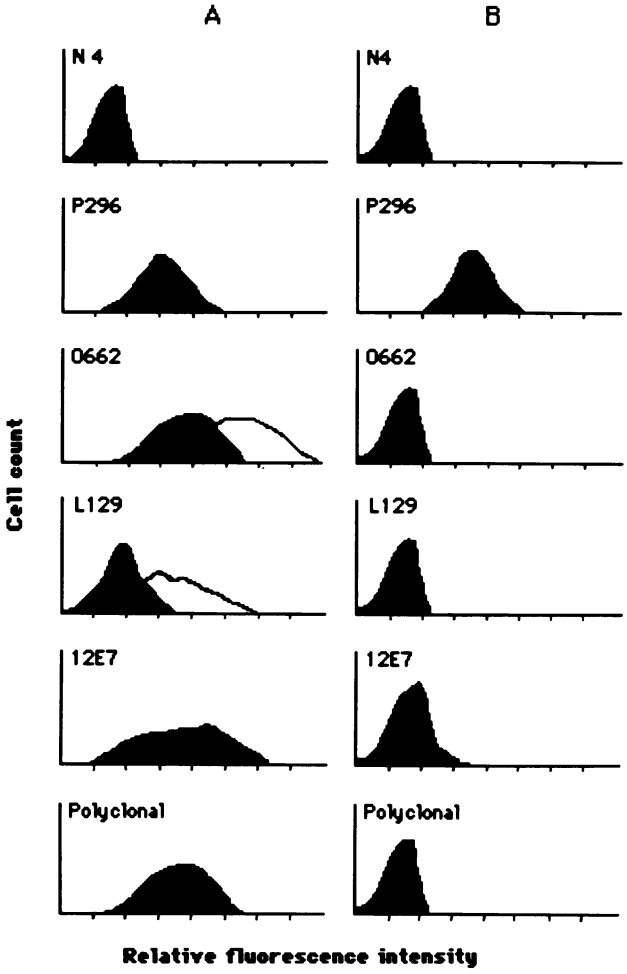

Fig. 4. Immunofluorescence histograms of E2 expression on human red blood cells. Red blood cells (10^6 cells) were labelled with a negative control (N4 mAb), a positive control (P296 mAb), with the 12E7, 0662 or L129 mAb and with the anti-E2 polyclonal antibody. (**A**) The Xg(a$^+$) red blood cells samples; (**B**) The Xg(a$^-$) red blood cells samples. Clear histograms represent the immunofluorescence obtained after neuraminidase treatment.

domain of the E2 protein is the extracellular region of the molecule.

Moreover, the E2 amino acid sequence was compared to protein sequences included in the NEWAT data bank and no significant homology to any known proteins was found. However, limited regions of similarity to collagen and collagen-like proteins were found and interestingly two regions could be delineated. Firstly, from residue 76 to residue 90 a region of homology containing five Gly-X-Y amino acid repeats, characteristic of collagens and collagen-like proteins, was detected. Secondly, from residue 45 to residue 62, E2 also has a high proline content, a property which could confer a marked globular structure.

Monoclonal antibodies 12E7, L129 and O662 define three different epitopes on the same 32 kd molecule

To confirm the relationship between the E2 molecule and the MIC2 gene product, immunoblotting experiments using various mAb were performed. The E2 molecule was purified from thymocytes by immunoaffinity using E2 mAb covalently linked to protein A-Sepharose CL-4B as descried elsewhere (Aubrit *et al.*, 1989). The purified material was subjected to Western blot analysis. Figure 3A shows that

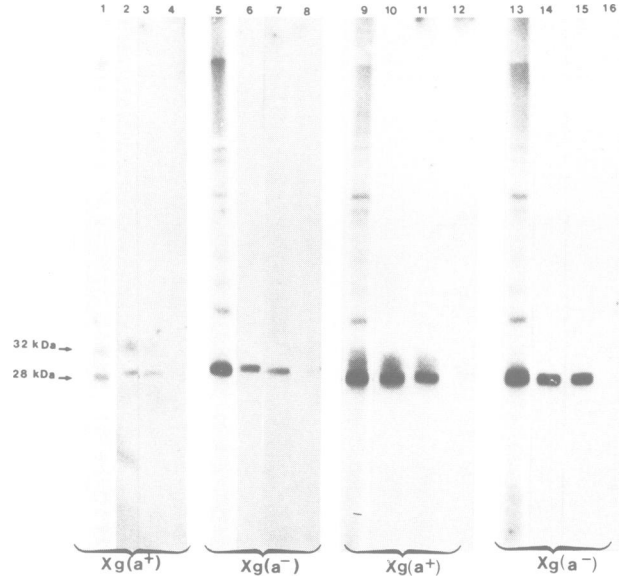

Fig. 5. Western blot analysis on red blood cells. Immunoblotting on untreated red blood cells (**lanes 1–8**) or on neuraminidase-treated red blood cells (**lanes 9–16**), from a Xg(a$^+$) donor (**lanes 1–4** and **9–12**) and a Xg(a$^-$) donor (**lanes 5–8** and **13–16**). Western blots were probed with the 12E7 mAb (**lanes 1, 5, 9, 13**), the 0662 mAB (**lanes 2, 6, 10, 14**), the L129 mAb (**lanes 3, 7, 11, 15**) or the irrelevant mAb (**lanes 4, 8, 12, 16**).

12E7 reacted with the purified E2 molecule to the same extent as the O662 mAb.

The next question was whether all three mAb recognized the same epitope on the E2 molecule. Competitive binding experiments were performed with biotinylated E2 mAb. It can be seen from Figure 3B that O662 and L129, two E2 mAb obtained in our laboratory, defined two different epitopes on the E2 molecule. Moreover this experiment revealed that 12E7 did not compete for binding with any E2 mAb tested (O662,L129). Thus the 12E7, L129 and O662 mAb define three different epitopes on the same 32 kd molecule.

Xg blood group and E2 expression on red blood cells

It has been reported that a quantitative polymorphism of 12E7 antigen expression on red blood cells is related to Xg blood group expression (Goodfellow and Tippett, 1981). Hence, we looked for the expression of 0662 and L129 epitopes on red blood cells of Xg(a$^+$) and Xg(a$^-$) individuals, compared to 12E7. Typical results obtained by immunofluorescence are shown in Figure 4. As described for 12E7 expression, a Xg(a$^-$) individual showed weak, or no expression of 0662 or L129 epitopes. This result was confirmed with a polyclonal antiserum obtained against the purified E2 molecule which did not react with erythrocytes from this donor. More surprising was the pattern of expression observed on Xg(a$^+$) red blood cells, on which the L129 epitope could not be detected. This result suggested that this epitope is either absent on red blood cells or masked because of sugar residues different from those described on lymphocytes (Aubrit *et al.*, 1989). To test this possibility, red blood cells were treated by neuraminidase and subsequently processed for immunofluorescence. Figure 4 shows that the L129 epitope can be detected if sialic acids are removed. Such a treatment did not modify the pattern of expression on Xg(a$^-$) red blood cells.

Table I. Ability of E2 mAb to inhibit the binding of human T cells to sheep (SE) or human (HE) erythrocytes.

Cells used in rosette assays	Antibody added				
	None	CD2	0662	L129	12E7
(Thymocytes+mAb)+ SE	100	00	43	21	85
+HE Xg(a$^+$)	55	02	27	14	51
+HE Xg(a$^-$)	45	01	22	18	40
Thymocytes+[HE Xg(a$^+$)+mAb]	47	45	42	42	46

Results are presented as % T cells binding erythrocytes.

In fact, the E2 molecule can be detected on lysates from Xg(a$^-$) red blood cells as shown by immunoblot experiments (Figure 5). Western blots performed on Xg(a$^+$) red blood cells revealed that 12E7 and 0662 recognized two major proteins of 32 and 28 kd (Figure 5, lanes 1 and 2), the latter probably corresponding to the unsialylated, fully glycosylated intracellular protein as suggested by our previous biochemical studies (Aubrit *et al.*, 1989). In contrast L129 only detected the 28 kd form (Figure 5, lane 3). In the same way, the 28 kd species was the only polypeptide to be revealed by the three mAb used on Xg(a$^-$) red blood cells. When sialic acids were removed before Western blot experiments (Figure 5, lanes 9–16), the only form to be detected was the 28 kd molecule irrespective of the mAb used or the donor tested. Thus a 32 kd molecule recognized by 12E7 and 0662 is present on Xg(a$^+$) red blood cells, the L129 epitope being undetectable on these cells because of sialic acids. In contrast, Xg(a$^-$) erythrocytes only expressed a 28 kd intracellular protein which cannot be detected on the cell surface.

Differential effect on rosette formation of mAb reacting with different epitopes of E2

The E2 molecule was first identified as a T cell surface antigen involved in rosette formation with sheep erythrocytes. Hence we have looked for the involvement of the 12E7 epitope in this phenomenon. Results are summarized in Table I. As previously described the 0662 and L129 mAbs strongly inhibited sheep erythrocyte rosette formation by T cells (57% and 79% inhibition respectively). Other mAb, 0667 (IgG1) and 0184 (IgG1), which define respectively the 0662 and L129 epitopes, were shown to block rosette formation with the same efficacity, thus demonstrating that the blocking effect was not related to the mAb isotype (data not shown). By contrast, the 12E7 mAb was unable to block rosette formation with sheep erythrocytes.

The blocking effect of the 0662 and L129 mAb was not restricted to rosette formation with sheep red cells but was also observed when rosette assays were performed with human erythrocytes. Thymocytes were tested in rosette assays with Xg(a$^+$) E2$^+$ erythrocytes, and with Xg(a$^-$) erythrocytes which express no E2 molecule on their surface, as described above. Rosettes between thymocytes and Xg(a$^+$) erythrocytes were inhibited by 0662 and L129 mAb as well as by CD2 mAb; as described with sheep erythrocytes, no inhibition was observed with the 12E7 mAb. T-cells bound normally to Xg(a$^-$) erythrocytes and the pattern of inhibition did not differ from that with Xg(a$^+$) red cells.

These observations led to the hypothesis that the inhibition of erythrocytes rosette formation was occurring at the level of the human T cells. To confirm this, red blood cells were pre-incubated with E2 mAb before rosette formation. As can be seen in Table I, no inhibition was exerted by the E2 mAb tested, thus indicating that the E2 molecule on the erythrocyte surface is not involved in rosette formation.

Pre-treatment of Xg(a$^+$) red cells with neuraminidase did not modify the percentage of T cells binding human erythrocytes, nor the level of inhibition observed with the L129 mAb (data not shown).

Discussion

The E2 cDNA isolated from a λgt11 library, prepared from thymic cells, by reactivity with E2 mAb has been sequenced. Several lines of evidence indicate that this clone corresponds to the gene encoding the E2 antigen. Most importantly, the N-terminal amino acid sequence encoded by the cDNA matches 12 to 14 residues of the N-terminal sequence determined for the purified E2 molecule. Furthermore, the size of the predicted polypeptide (mol. wt 16 710) is in good agreement with the value determined by SDS–PAGE for the non-glycosylated polypeptide (~ 18 000) derived by *O*-glycanase treatment (Aubrit *et al.*, 1989). Finally, transcription of the gene is coincident with cell surface expression of the antigen. Thus 1.2 kb transcripts were detected in thymocytes, peripheral blood cells, T-cell lines and monocytic line, and undetectable in the B-cell line Daudi which did not show reactivity with the 0662 and L129 mAb.

The amino acid sequence reveals that E2 appears to be a transmembrane glycoprotein sharing limited regions of similarity with a class of human 'collagen-like' proteins. These proteins have a similar structural organization and are characterized by Gly-X-Y amino acid repeats where Y is frequently a proline hydroxylated post-translationally. These features are involved in the subunit assembly of collagen-like molecules in which three polypeptide chains are non-covalently associated via an N-terminal collagen helix (Bornstein and Sage, 1986). However, while E2 has a high proline content, no proline residues are found in the five Gly-X-Y amino acid triplets. Definitive identification of E2 as a collagen-like molecule requires additional evidence that E2 can form non-covalently associated trimers.

The nucleotide sequence identifies E2 as the product of the MIC2 gene which is the only pseudoautosomal gene so far described in man. In fact, it has been demonstrated that the human sex chromosomes share a pair of related genes, MIC2X and MIC2Y (Goodfellow *et al.*, 1986), encoding the cell surface antigen recognized by the 12E7 mAb (Levy *et al.*, 1979). MIC2X is located at the end of the short arm of the human X chromosome (Xp22.3-Xpter) (Buckle *et al.*, 1985) and the homologous gene, MIC2Y, is in the euchromatic region of the human Y chromosome (Yq11-Ypter) (Goodfellow *et al.*, 1983).

The 12E7 mAb which recognized the purified E2 molecule, and the 0662 and L129 mAb, define three different epitopes on the molecule. The detection of these epitopes is different on red blood cells compared to lymphocytes. Thus, the results presented in this paper first confirmed a quantitative polymorphism of E2 expression on human erythrocytes, involving the blood-group-Xg phenotype (Goodfellow and Tippett, 1981; Latron *et al.*, 1987). Secondly, they demonstrate that while the 32 kd cell surface E2 molecule is undetectable on Xg(a$^-$) erythrocytes, these cells expressed the 28 kd intracellular protein. Our

previous biochemical studies (Aubrit *et al.*, 1989) suggested that this 28 kd molecule corresponds to the unsialylated fully glycosylated protein and thus raise the possibility that Xg(a$^-$) women do not have the capacity to export the E2 molecule to their cell surface and this is clearly due to a defect of sialylation. Thirdly, it is shown that the sialylation pattern of the molecule on Xg(a$^+$) red blood cells prevent the detection of the L129 epitope.

All these results propose for the first time a role for the only pseudoautosomal gene actually described. The E2 antigen is not only actively synthesized by T cells and monocytic cells, but also found on extra-haematopoietic cells. Our results demonstrate that E2 is clearly involved in adhesion processes and especially in spontaneous rosette formation with erythrocytes, including autologous red cells. The present data indicate that while E2 is present on both types of cells, T and red cells, the binding of human erythrocytes to T lymphocytes involves the E2 T cell surface molecule. Moreover those results suggest that a particular region of the molecule, including among others the 0662 and L129 epitopes, is implicated in this adhesion phenomenon. We are now investigating more precisely, by epitope mapping and site-directed mutagenesis, the regions of the molecule involved in this interaction. At the present time, we do not know how E2 functions in rosette formation. Nevertheless, E2 could function as a ligand for one of the two red blood surface proteins we have described, i.e. H19 or H110-220, or another, as yet unknown surface molecule. The fact that E2 is involved in rosette formation strongly supports the view that it is also involved in T-cell adhesion phenomenon, as exemplified by the CD2−LFA3 interaction. Moreover, its broad cellular distribution suggests that E2 is potentially involved in adhesion to many other cell types. The elucidation of the quaternary structure of the molecule—we do not know if E2, like collagen-like proteins, forms non-covalently associated trimers—and the research of its putative ligand will give further clues to T-cell adhesion processes.

Materials and methods

Cells

Human thymi were obtained from children undergoing cardiac surgery; single thymocyte suspensions were prepared by gently teasing cells from thymic samples. Peripheral blood lymphocytes were obtained from healthy donors by leukophoresis and were prepared as previously described (Bernard *et al.*, 1982). Xg(a$^+$) and Xg(a$^-$) human erythrocytes were obtained from healthy donors. Human and sheep erythrocytes were prepared by removing the buffy coat after centrifugation and washing three times in phosphate-buffered saline (PBS). All cell lines were grown in RPMI 1640 supplemented with 10% fetal calf serum, 100 U/ml of pencillin and 50 μg/ml of streptomycin. HDMAR is a T leukaemia cell line (Ben Bassat *et al.*, 1980) and Daudi is a Burkitt lymphoma cell line (Klein *et al.*, 1968).

Monoclonal and polyclonal antisera

The two E2 mAb used, i.e. 0662 (IgG3) and L129 (IgM), were prepared as previously described (Bernard *et al.*, 1988). Briefly, they were obtained after immunization of Biozzi's high responder mouse strain with thymocytes and selected for their capacity to inhibit rosetting between T-cells and sheep red blood cells. The 12E7 mAb (IgG1) was kindly provided by Dr R.Levy (Levy *et al.*, 1979). The N4 (Bernard *et al.*, 1987) and P296 (Groux *et al.*, 1989) mAb were obtained in our laboratory. The N4 mAb defines a 14 kd protein present on sheep erythrocyte surface and the P296 mAb defines a 19 kd protein present on the human erythrocyte surface. Purified mAb were biotinylated following a technique previously described (Bernard *et al.*, 1982).

λgt11 cDNA library screening

A cDNA library, constructed in the phage vector λgt11 and prepared from human thymocytes was obtained from Clontech Laboratories (Genofit). *Escherichia coli* strain Y1090 was infected with γt11 cDNA library and plated for 3 h at 42°C and overnight at 37°C. Nitrocellulose discs which were placed in 10 mM IPTG and air-dried, were placed in contact with plaques for 3 h at 37°C to induce synthesis of and bind β-galactosidase fusion protein. The nitrocellulose filters were then processed in an immunoscreening assay using anti-E2 purified monoclonal antibodies and a ^{125}I-labelled F(ab') fragment of goat anti mouse Ig (Amersham) as described (Bernard *et al.*, 1988).

DNA sequencing

The insert from cDNA clone BC1 was subcloned into the *Eco*RI site of pUC8, then appropriate restriction fragments were isolated and subcloned into the *Sma*I site of the M13 vector mp18. Single-stranded templates were prepared and sequenced using the dideoxy chain terminator procedure (Sanger *et al.*, 1977). The entire sequence of the E2 polypeptide chain was determined on both strands. Compressions in GC-rich regions were resolved by replacing dGTP with ITP in some sequencing reactions.

Northern blotting

The cells were lysed in a 6 M guanidium isothiocyanate solution and the RNA was isolated on a 5.7 M CsCl gradient. Poly (A$^+$) mRNA was selected with oligo(dT)-cellulose (Pharmacia). RNA was denaturated, electrophoresed on 1.2% agarose gels and transferred to nylon membranes (Amersham) in 20 × SSC. The filters were hybridized with the insert of clone BC1 labelled using multiprime DNA labelling system (Amersham). The hybridization was carried overnight at 62°C in 6 × SSC, 0.01 M EDTA, 5 × Denhardt's, 0.1% SDS and 100 μg/ml denaturated salmon sperm DNA. The filters were washed first for 1 h at 62°C in 2 × SSC, then for 15 min at 62°C in 1 × SSC.

Biochemical analysis of the E2 purified molecule

The E2 molecule was purified from thymocytes as described elsewhere (Brown *et al.*, 1987; Aubrit *et al.*, 1989). Western blotting on red blood cells obtained from healthy donors were lysed by brief incubation in 0.01 M Tris−HCl (pH 7.8) containing 5 mM EDTA, 5 mM iodoacetamide and 50 μM phenylmethylsulfonyl fluoride (PMSF) as described elsewhere (Bernard *et al.*, 1987). Lysates were electrophoresed on SDS−PAGE under reducing conditions then electrophoretically transferred to nitrocellulose. Nitrocellulose sheets were next incubated with mAb (10 μg/ml) and [^{125}I]F(ab') anti-mouse Ig fragment before autoradiography.

Immunofluorescence assays

Cells were stained with saturating amounts of mAb and staining was revealed by affinity-purified fluorescein isothiocyanate-conjugate goat anti-mouse Ig as described previously (Gelin *et al.*, 1986). Cells were analysed by using cytofluorometry (Ortho-Becton Dickinson, System 50). The negative control used was taken from the same cell preparation, but stained with ascites containing an irrelevant antibody. In competitive binding experiments target cells were thymocytes. They were incubated with the first mAb (full range of dilution was investigated, up to 1 mg/ml) for 30 min at room temperature. Then the second antibody, coupled to biotin, was added and the fixation revealed by fluorescein conjugate avidin as described (Bernard *et al.*, 1982).

T-cell E binding (rosette) assay

Detailed procedures for neuraminidase treatment, rosette formation and rosette inhibition assays have been described (Bernard *et al.*, 1982). T cell preparations were incubated with mAb (50 μg/ml) for 15 min at 22°C. Next, the erythrocyte suspension was added and the mixture was centrifuged and incubated at 4°C. Rosettes were counted as thymocytes tightly bound to at least three red cells and 300 mononuclear cells were counted in each test. Each experiment was performed at least five times. In some experiments, red cells were pre-incubated with mAb preparation and washed three times before allowing the formation of rosettes.

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