

# Mutational Analysis of the CD2/CD58 Interaction: The Binding Site for CD58 Lies on One Face of the First Domain of Human CD2

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## Summary

The adhesion interaction between the immunoglobulin superfamily molecules CD2 and CD58 (lymphocyte function-associated antigen 3) plays an important role in T cell and natural killer cell interaction with various antigen-presenting and target cells. Determination of the solution structure of rat CD2 domain 1 has allowed a model of human CD2 domain 1 to be generated, and a series of mutants based on this model have been made. Residues of domain 1 of human CD2 predicted to be solvent exposed were substituted with the equivalent residues present in the rat CD2 molecule. The ability of these mutants to mediate rosetting with human and sheep erythrocytes was studied. Results show that the binding site of CD2 for both human and sheep CD58 maps to the  $\beta$  sheet containing  $\beta$  strands CC'C''F and G. Residues K34 and E36 in  $\beta$  strand C, R48 and K49 in  $\beta$  strand C', and K91 and N92 in the loop connecting  $\beta$  strands F and G are shown to be critical in the interaction. The data support the proposition that the interaction between CD2 and CD58 involves the major  $\beta$  sheet face of CD2.

The interaction between the CD2 glycoprotein on T lymphocytes and the widely expressed CD58 (LFA-3) molecule serves an important role in the T cell immune response. CD2/CD58-mediated intercellular adhesion has been shown to have a role in the processes of cell-mediated cytotoxicity, antigen- and mitogen-induced T cell proliferation, and lymphokine production (1–4). The interaction was first identified as that supporting rosette formation between T lymphocytes and sheep or human erythrocytes (5–7). Particular combinations of mAbs directed against different regions of CD2 induce signal transduction events that stimulate T cell proliferation, as well as T and NK cell effector functions (8–10). These effects can also be obtained by incubation of the cells with cell-bound or soluble CD58, or phorbol esters plus certain anti-CD2 mAbs (11–14). The cytoplasmic domain of CD2 is essential for the signaling events (15, 16). Signal transduction by CD2 requires the presence of a functional TCR/CD3 complex on T cells and the CD16/ $\zeta$  or Fc $\epsilon$  receptor  $\gamma$  complex on NK cells (17, 18). It has recently been demonstrated that CD2 belongs to a T cell activation complex formed by

the association of the TCR, CD3, CD4, or CD8 and CD5 surface molecules and intracellular tyrosine kinases (19).

The structure of the NH<sub>2</sub>-terminal domain of rat CD2 was initially determined by nuclear magnetic resonance (NMR)<sup>2</sup> spectroscopy (20), and more recently the crystal structure of the complete extracellular part of rat CD2 has been reported (21). Both structures have confirmed earlier predictions that the extracellular region of CD2 consisted of two immunoglobulin superfamily (IgSF) domains, an NH<sub>2</sub>-terminal V-like domain, lacking the usually conserved disulphide bridge, and a membrane-proximal C-like domain (22, 23).

Two ligands for human CD2 have been identified so far: the CD58 molecule (3) and the complement regulatory protein, CD59 (24). A homologue of the CD58 antigen has not yet been found in rodents. However, the CD48 molecule has been shown recently to be a ligand for CD2, both in the mouse (25) and rat (26). CD2 and its ligands, CD58 and CD48, are closely related molecules that contain two IgSF domains (27). They form a subgroup within the IgSF and their genes are linked in the genome, indicating that they may have evolved by gene duplication (28).

<sup>1</sup> Alan F. Williams died on April 9, 1992.

<sup>2</sup> Abbreviations used in this paper: IgSF, immunoglobulin superfamily; NMR, nuclear magnetic resonance.

Experiments using recombinant truncated forms of human CD2 showed that the NH<sub>2</sub>-terminal domain of CD2 contained the binding site for CD58 (29). This was consistent with a random mutagenesis analysis that demonstrated that certain residues of domain 1 of CD2 were involved in ligand binding (30). Based on the NMR structure of domain 1 of rat CD2, we predicted that the residues of human CD2 shown to be involved in the interaction with CD58 (30) were located in  $\beta$  strand C' and in the region between  $\beta$  strands F and G, and it was proposed that the face of the CC'C''FG  $\beta$  sheet of human CD2 might be involved in ligand binding (20). We have investigated this issue by producing a panel of mutants covering many of the surface-exposed residues of domain 1 of human CD2. We exploited the fact that rat CD2 does not form rosettes with sheep or human erythrocytes and substituted residues of human CD2 by the corresponding amino acid present in rat CD2. Here we report that all the mutations that disrupt the CD2/CD58 interaction lie in the CC'C''FG  $\beta$  sheet of human CD2. The charged side changes of solvent-exposed residues on  $\beta$  strands C and C', and on the FG loop, appear to play a critical role in the CD2/CD58 interaction.

## Materials and Methods

**Generation of Human CD2 Mutants.** CD2 mutants were produced using a two-step PCR method adapted from that described in reference 31, using Taq (Boehringer Mannheim Biochemicals, Indianapolis, IN) or Vent (New England Biolabs Inc., Beverly, MA) DNA polymerase. Briefly, in the first PCR round, a common primer, introducing an XbaI restriction site at the 5' end of the human CD2 cDNA, and a series of 21 3' primers were used to amplify fragments of domain 1 of human CD2. Each 3' primer was specific for a particular strand or loop of CD2 domain 1 and contained nucleotide changes such that the PCR product was mutated with respect to the wild-type template. In a second PCR reaction, the gel-purified (QIAGEN Inc., Chatsworth, CA) double-stranded products of the first PCR amplification were used as primers along with a common antisense primer adding an EcoRI site in the 3' untranslated region of CD2. The final products obtained, CD2 mutants flanked by XbaI and EcoRI restriction sites, were gel extracted, subcloned into the pJFE14 expression vector (32), and used for transient transfection of COS-7 cells (33). The pJFE14 vector, kindly provided by John F. Elliott (University of Alberta, Edmonton, Canada), uses the same cloning strategy as CDM8 but contains a pBR322 origin of replication, an ampicillin resistance gene, and a human T lymphocyte virus type 1 (HTLV-1) promoter. The template used in both PCR reactions was the linearized full-length cDNA of human CD2 in Bluescript KS<sup>-</sup>, a gift from D. Wotton (Imperial Cancer Research Fund, London, UK). The wild-type human CD2 was released from Bluescript KS<sup>-</sup> with EcoRI, blunt ended by treatment with Klenow (Amersham Corp., Arlington Heights, IL), and ligated into a blunt-ended pJFE14 vector. Rat CD2 in CDM8 provided by S. Mallett (MRC Cellular Immunology Unit) was used as a control. Mutants in pJFE14 were sequenced using the chain termination method.

**Transfection of COS-7 Cells.** COS-7 cells were transfected using the DEAE-dextran procedure (34). Briefly, 1–2 × 10<sup>7</sup> cells were plated and cultured overnight in RPMI 1640 (Gibco, Paisley, UK) supplemented with 10% FCS, 2 mM glutamine, and antibiotics (50 mg/ml streptomycin sulphate, 100 mg/ml kanamycin, and 50

mg/ml penicillin G), and transfected with 3–5  $\mu$ g of plasmid DNA of the wild-type or mutant CD2. After 24 h cells were plated into two to three 60-mm petri dishes. Expression of the constructs was analyzed by indirect immunofluorescence and flow cytometry 2 d posttransfection, and 24 h later the remaining cells were used in the rosetting assay described below.

**Anti-CD2 mAbs and Immunofluorescence Analysis of Transfected COS-7 Cells.** The mAbs used in this study were: F92-3A11, CLB-T11/1, 95-5-49, and OCH-217 (a gift from F. Gotch, Institute of Molecular Medicine, Oxford, UK) (35), and RFT-11 (36), kindly provided by M. H. Brown (MRC Cellular Immunology Unit). Rat CD2 was detected with the MRC OX-34 mAb. MRC OX-21, anti-human C3b inactivator, was used as negative control and W6/32 anti-HLA class I as a positive control. Transfected COS-7 cells were detached using PBS containing 5 mM EDTA, and indirect immunofluorescence staining was performed as described (16). Labeled cells were analyzed on a FACScan<sup>®</sup> (Becton Dickinson & Co., Mountain View, CA).

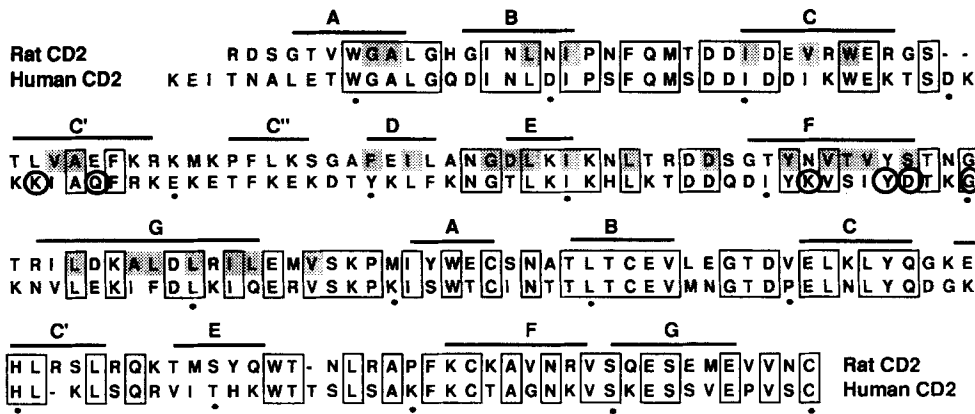
**Rosetting Assays.** Human erythrocytes were obtained from blood of healthy donors, washed three times in PBS, and treated with *Vibrio cholera* neuraminidase (0.025 U/10<sup>7</sup> cells; Calbiochem-Novabiochem Corp., La Jolla, CA) for 45 min at 37°C. The cells were then washed twice with PBS and resuspended as a 20% solution (vol/vol) in PBS. Sheep erythrocytes in Alsevers solution (Becton Dickinson & Co.) were washed four times in PBS and resuspended at 20% (vol/vol) in PBS. Rosetting assays were performed as described (34). Transfected COS-7 cells in 6-cm petri dishes were washed twice with PBS and 2 ml of PBS/5% FCS was added to each plate, followed by 0.5 ml of the erythrocyte suspension. Plates were incubated at 4°C for 1 h and the nonadherent erythrocytes removed by several gentle washes with PBS.

**Molecular Modeling.** A model of domain 1 of human CD2 was generated from the NMR structure of rat CD2 (20) in the homology modeling facility of the QUANTA molecular graphics package (Molecular Simulations Inc., Burlington, MA). The torsional angles of residues with differing side chains were adjusted to maintain similar orientations in the model. The insertion of two residues in the CC' loop was performed by searching a library of protein backbone fragments from high-resolution protein crystal structures. The stereochemistry and nonbonded contacts of the model were then optimized by two rounds of molecular dynamics at 300K and energy minimization.

## Results

**Design of Human CD2 Domain 1 Mutants.** The three-dimensional structure of rat CD2 has been determined both by NMR spectroscopy (domain 1) (20) and x-ray crystallography (21). Assignment of the  $\beta$  strands for human CD2 was made based on an alignment of the human CD2 sequence with that of rat CD2 (Fig. 1; numbering, indicated by dots, refers to the human CD2 sequence). A previous mutagenesis study predicted, based on an alignment of human CD2 and the Ig  $\kappa$  chain, that the adhesion interaction between CD2 and LFA-3 would involve the regions of CD2 corresponding to the Ig hypervariable regions, and will mimic an antibody-antigen interaction (30).

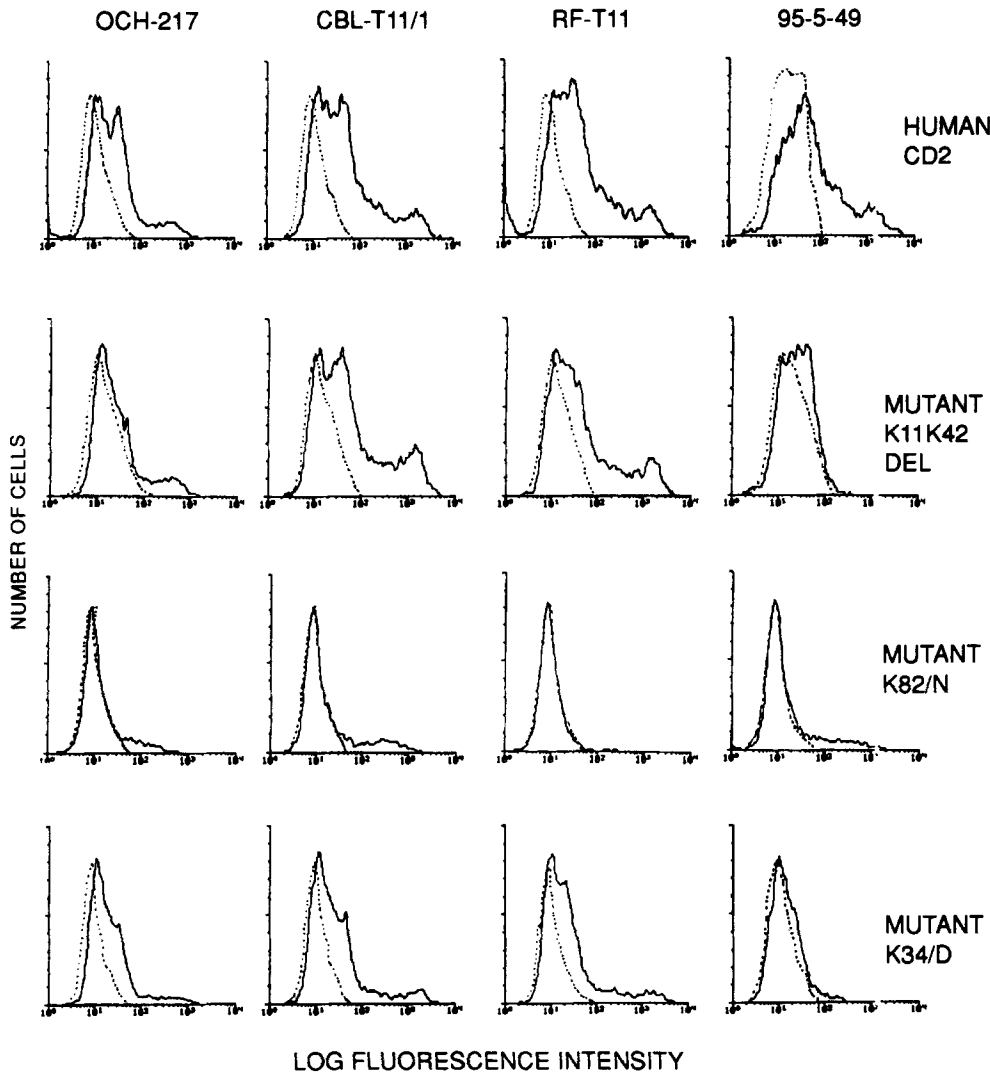
However, in light of the new alignment it was evident that the subset of residues that had been shown both to affect CD58 binding (30) and to be solvent exposed (20), indicated by circles in Fig. 1, were located in  $\beta$  strand C' and in the



**Figure 1.** Alignment of rat and human CD2 extracellular region sequences, with the positions of the  $\beta$  strands determined from the rat CD2 structure (20, 21) marked by a thick overline. (●) Every 10th residue. Numbering refers to the amino acid position of human CD2 with respect to the mature NH<sub>2</sub> terminus of the molecule (48). Residues identical between rat and human CD2 are boxed and those previously shown to be involved in the CD2/CD58 interaction (30) are circled. Solvent inaccessible residues of rat CD2 domain 1 are shaded.

region between  $\beta$  strands F and G, suggesting that the CD2/CD58 interaction might be mediated through the  $\beta$  sheet of CD2 domain 1 containing strands CC'C''F and G. A model of human CD2 domain 1, generated from the structure of rat CD2 domain 1, was used to design a panel of

21 mutants to identify the residues of human CD2 that affect binding to CD58 and hence estimate the extent of the site of interaction. Amino acids in both  $\beta$  sheets of human CD2 at positions predicted to be solvent exposed were mutated because these residues were the most likely to be involved



**Figure 2.** Expression of human CD2 epitopes in wild-type and mutant CD2. COS-7 cells transfected with human CD2 cDNA in pJFE14 or the indicated mutant were stained with anti-CD2 mAbs directed to domain 2 (OCH-217) or domain 1 (CLB-T11/1, 95-5-49 and RFT-11) of human CD2 (solid lines) or the OX-21 mAb (dotted lines) as negative control.

in ligand interaction. We initially determined that rat CD2 did not rosette with sheep or human erythrocytes, and then, to minimize the possibility of major conformational changes in human CD2, we substituted human residues with the corresponding amino acid present in rat CD2. Where the residues in rat and human were identical a nonconservative substitution was used. Mutations were introduced at many of the out-pointing positions in each strand of the two  $\beta$  sheets and in the loops connecting the  $\beta$  strands (Fig. 1).

**Expression of Human CD2 Mutants in COS-7 Cells.** The constructs encoding the wild-type and mutant CD2 in the pJFE14 eukaryotic expression vector were transiently expressed in COS-7 cells. Expression of the mutants was analyzed by flow cytometry using a series of anti-CD2 mAbs. The mutants were stained with at least one antibody binding to domain 1 of human CD2 (F92-3A11, CLB-T11/1, 95-5-4, and RF-T11), and the OCH-217 antibody that binds an epitope on domain 2 of human CD2 and is weakly expressed in resting T cells and transfected COS-7 cells (30). In most cases, and always when a mutant failed to bind to one of the antibodies, expression was detected with three or more mAbs to rule out structural distortion of the molecule or its retention inside the cell. FACS<sup>®</sup> profiles of COS-7 cells expressing human CD2 and several mutants stained with anti-CD2 mAbs are shown in Fig. 2, and the pattern of antibody binding of the CD2 mutants is summarized in Table 1.

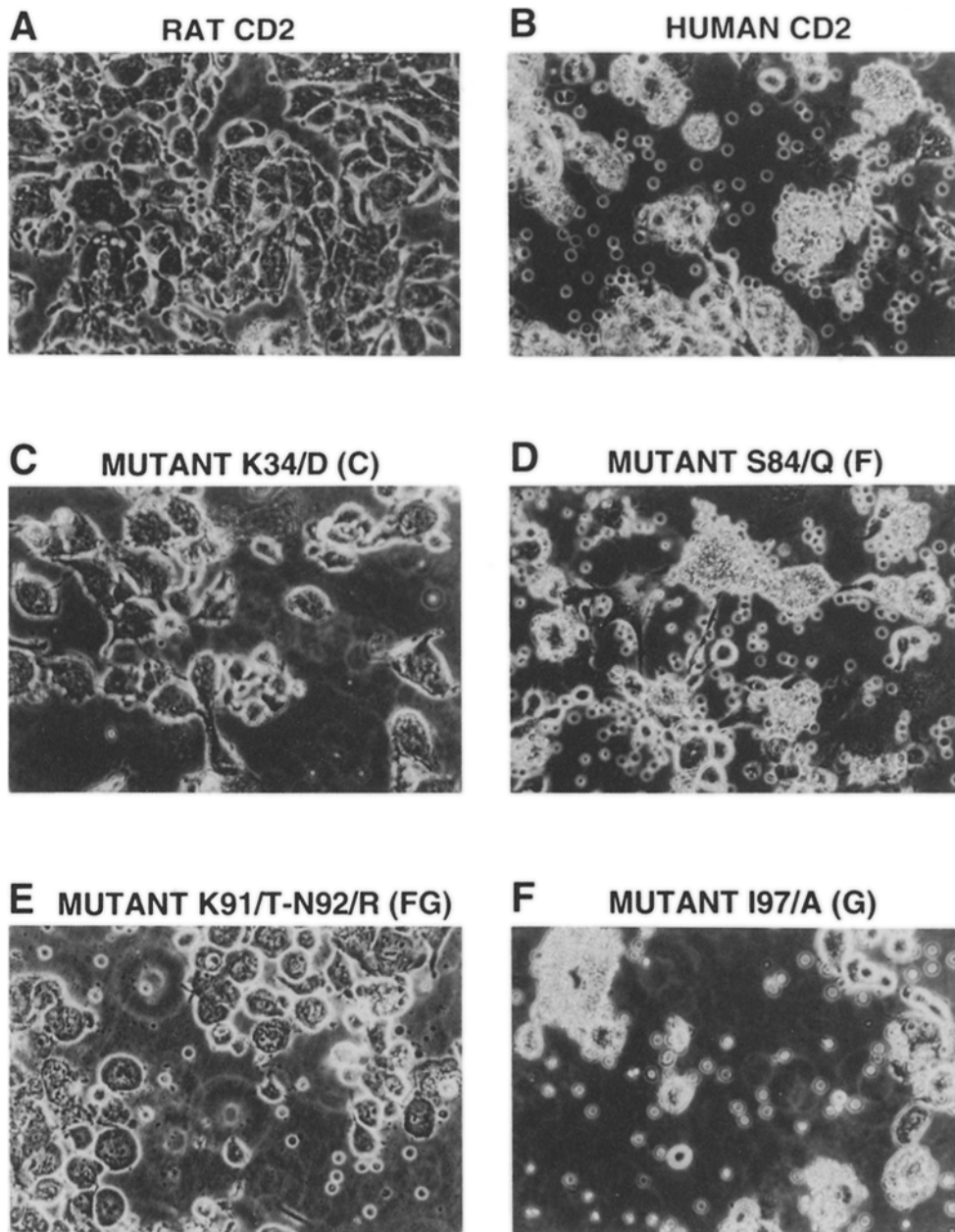
**Analysis of the Ability of Mutant CD2 Molecules to Interact with CD58 by E-Rosetting.** The ability of the mutated forms of CD2 to mediate CD58 binding was measured using an E-rosetting assay (34). It has been previously shown that anti-CD58 mAbs completely inhibit E-rosetting of CD2-expressing cells (6) and that mutations of CD2 that abolish E-rosetting also disrupt CD58 binding (12, 37). Monolayers of COS-7 cells expressing wild-type or mutant human CD2 were screened for rosette formation with sheep and neuraminidase-treated (Neu<sup>+</sup>) human erythrocytes. COS-7 cells transfected with rat CD2 in CDM8 were unable to form rosettes with sheep or Neu<sup>+</sup> human erythrocytes and were used as a negative control (Fig. 3 A). There was a clear distinction between the mutants that supported rosette formation and those that did not, as illustrated in Fig. 3. These data are summarized in Table 2. Substitutions on  $\beta$  strands B (N18/T+D20/N [17]) and D (K61/E[56]) had no effect on the rosetting ability of CD2. Mutations of  $\beta$  strand E were not performed because most of its residues are buried and therefore unlikely to establish interactions with the ligand (Fig. 1). All the mutations that abolish E-rosetting are located on  $\beta$  strands belonging to the CC'C''FG face of CD2. These include substitution of the charged residues K34 and E36 on  $\beta$  strand C (Fig. 3 C) and a double mutation in which the positions of residues R48 and K49 on strand C' were inverted to KR. Consistent with previous results (30), a mutant in which residue K82 on strand F was changed to an N, present in rat CD2, exhibited no E-rosetting activity. This substitution generates the sequence NVS (residues 82-84) in human CD2, which constitutes an N-glycosylation signal. Interestingly, it has been demonstrated that rat CD2 has an N-linked carbohydrate attached to residue N77 (21), the equivalent posi-

Table 1. Summary of the Binding of anti-CD2 mAbs to CD2 Mutants

mAb	N18/T D20/N	K34/R* or D	E36/R	T38/G	K37/R K41K42 DEL	R48/K K49/R DEL	E50/K E51/M E52/K	F54/L K57/S	K61/E	I80/T	K82/N	S84/T* or Q	S84/Q	K82/N	K89/N	N92/R	E95/D	I97/A
RF-T11	+	+	+	+	+	+	+	+	+	+	-	+	+	+	±	+	+	+
F92-3A11	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CLB-T11/1	ND	ND	+	+	+	ND	ND	ND	ND	ND	+	ND	+	+	±	+	+	ND
95-5-49	ND	ND	-	±	-	ND	ND	ND	ND	ND	+	ND	+	+	+	+	+	ND
OCH-217	+	+	+	+	±	+	+	+	+	+	+	+	+	+	+	+	+	+

+, binding of mAb identical to that of wild-type human CD2; ±, decreased binding, in comparison with that of human CD2; -, no mAb binding. The nomenclature of the mutants is as in Table 2, but the position of the introduced residue in the rat CD2 sequence is omitted.

\* Results of two different mutations of the same residue.



**Figure 3.** Phase contrast microscopy of E-rosetting of transfected COS-7 cells. Cells transfected with constructs for rat CD2, human CD2, or mutant CD2 molecules were incubated with Neu<sup>+</sup> human erythrocytes for 1 h at 4°C, and the nonadherent erythrocytes washed off. Rosettes were examined under light microscope. The location of the mutations in the  $\beta$  strands or loops of CD2 domain 1 are indicated in parentheses.

tion to residue K82 in human CD2 (Fig. 1). We therefore reasoned that attachment of a carbohydrate structure at the newly created N-glycosylation site on human CD2, when expressed in COS-7 cells, could be responsible for the disruption of the CD2/CD58 interaction. To test this possibility, mutant S84/Q, which had a normal rosetting ability (Fig. 3 D), was further mutated, changing K82 to N, thus preventing the formation of an N-glycosylation signal. The double mutant K82/N[77]+S84/Q formed rosettes with Neu<sup>+</sup> human erythrocytes (Table 2), which strongly suggests that residue K82 is not directly involved in CD58 binding and that the lack of rosetting ability of the K82/N[77] mu-

tant is due to the attachment of a carbohydrate molecule to the face of human CD2 involved in the interaction with CD58.

A double mutation affecting the loop between strands F and G and the beginning of strand G (K91/T[86]+N92/R[87]) prevents the formation of E-rosettes (Fig. 3 E), confirming the importance of this region of CD2 in CD58 binding (30). The contribution of  $\beta$  strand G to the CD2/CD58 interaction was further analyzed in two mutants, E95/D[90] and I97/A[92], but both had an unaltered E-rosetting ability (Fig. 3 F). The involvement of other loops connecting  $\beta$  strands on the CD2/CD58 interaction was analyzed in several mutants. A deletion mutant in which two lysine residues

**Table 2.** Panel of Human CD2 Mutants and Their E-rosetting Ability

Mutant	Strand/Loop	E-rosetting
N18/T	B	+
D20/N[17]		
D32/E[29]	C	+
K34/R[31]	C	+
K34/D	C	-
E36/R	C	-
K37/R[34]	C/C'	+
T38/G[35]		
K41K42 DEL	C/C'	+
R48/K[43]	C'	-
K49/R[44]		
E50/K[45]	C'/C''	+
K51/M[46]		
E52/K[47]		
F54/L	C''	+
K55/L[50]	C''	+
E56/K[51]		
K57/S[52]		
K61/E[56]	D	+
I80/T[75]	F	+
K82/N[77]	F	-
S84/T[79]	F	+
S84/Q		+
K82/N[77]	F	+
S84/Q		
K89/N[84]	F/G	+
K91/T[86]	F/G	-
N92/R[87]		
E95/D[90]	G	+
I97/A[92]	G	+

Mutants are noted by the one-letter code of the amino acid(s) substituted and their position(s) in the human CD2 sequence, followed by a slash, and the residue(s) introduced with its position(s) in the rat CD2 sequence in brackets when applicable. A mutation involving the deletion of two amino acids is noted (DEL). Rosetting identical to that of wild-type CD2 is indicated (+), and no detectable rosetting (-).

(K41K42) were removed from the CC' loop and a double mutant (K37/R[34]+T38/G[35]), affecting the CC' loop, showed a wild-type E-rosetting ability (Table 2), suggesting that this region is not required for CD58 binding. In another mutant the loop connecting strands C' and C'' was almost entirely substituted by the corresponding rat CD2 residues (E50/K[45]+K51/M[46]+E52/K[47]), and the resulting protein did not show an impairment of its E-rosetting ability. Furthermore, the replacement of the residues forming  $\beta$  strand C'' for either their rat CD2 equivalents (K55/L[50]+E56/K[51]+K57/S[52]), or in the case of residue 54, substitution by L (F54/L) had no effect on erythrocyte binding. From these results we conclude that the CC' and C'C'' connecting loops and the C''  $\beta$  strand, although located on the CD58 binding face, are unlikely to be critical in the interaction between CD2 and CD58.

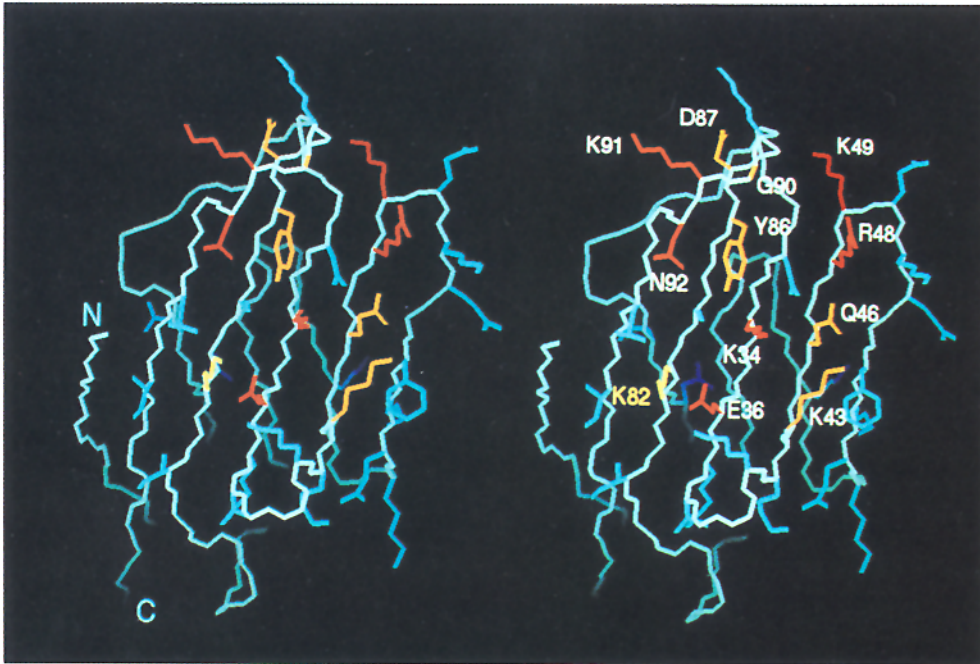
A model of human CD2 domain 1 based on the NMR solution structure of rat CD2 domain 1 (20) is shown in Fig. 4. The human CD2 model exhibits a backbone fold essentially identical to that of the rat CD2 structure, with no major rearrangements of internal residues, which suggests that the model should serve as a good basis for the interpretation of the mutagenesis results. Fig. 4 A summarizes the mutations described in this and other studies of CD2/CD58 interactions. In this model the binding site of human CD2 for CD58 is represented by showing amino acids involved in the CD58 interaction in red (present study) and orange (reference 30). In blue are shown residues that do not affect the CD2/CD58 interaction Fig. 4 B shows a space-filling representation of the CD2 model and shows the residues, that when mutated, affect the rosetting function of CD2 cluster together in a surface patch that encompasses the "top" half of the F, C, and C'  $\beta$  strands as well as residues in the F-G loop.

## Discussion

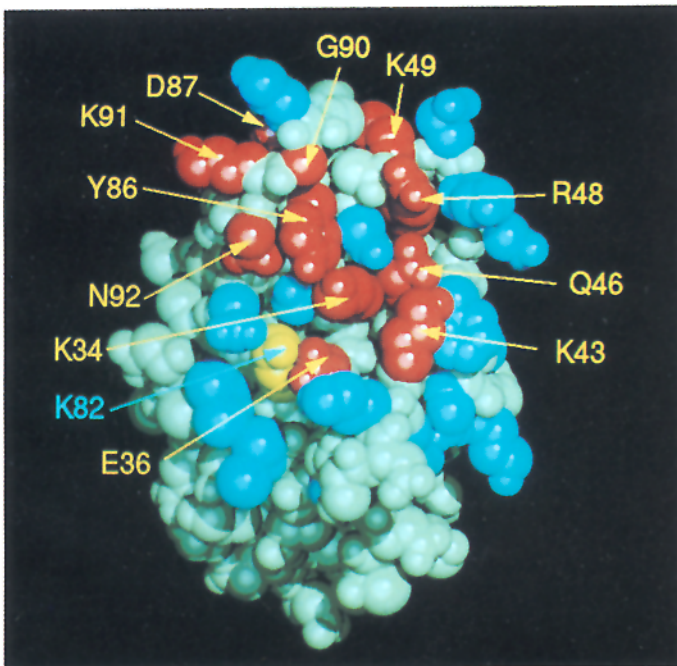
Our findings demonstrate that all residues affecting sheep and human erythrocyte binding to CD2 are located in the five-stranded  $\beta$  sheet of domain 1 of human CD2, with contact residues being restricted to strands C and C' and the loop-connecting strands F and G. Mutations in the opposite face of the domain ( $\beta$  strands B and D) do not interfere with the interaction. Although it has recently been shown that CD59, another molecule expressed on human erythrocytes, is able to bind human CD2 (25, 38), other studies have shown that the E-rosetting of CD2-transfected COS cells is completely blocked by anti-CD58 mAbs (6), making it unlikely that the CD2/CD59 interaction is measured by this system.

The relative contribution of the different regions of the interaction face of CD2 is variable. The five-stranded  $\beta$  sheet of CD2 domain 1 is a highly charged surface, and a number of the charged residues are shown to be involved in the interaction. In particular, charged residues K34 and E36 on strand C, and R48 and K49 on strand C', are important. The replacement of residue K34 for its equivalent, R31, in rat CD2, a conservative change that does not alter the charge, has no effect on the CD58 binding. In contrast, when residue K34 is substituted for an acidic residue, D, CD58 binding is com-

A



B



**Figure 4.** (A) Stereo view of the model of human CD2 domain 1 based on the NMR structure of rat CD2. The backbone fold is indicated in light blue, with the major CC'C''FG  $\beta$  sheet to the front. Amino acid side chains of residues that have been mutated in either this or previous studies are also shown. All these side chains are exposed to the solvent and their precise conformation is indeterminate. Residues previously shown to be involved in binding CD58 (reference 30) are shown in orange. The other side chains are residues that have been mutated in this study. Residues that when mutated do not affect the CD2/CD58 interaction are shown in blue. The side chains of residues that when mutated knock out the rosetting function of CD2 are shown in red. The side chain of lysine-82, which when mutated to asparagine becomes a consensus N-linked glycosylation site, is shown in yellow. (B) Space-filling representation of the complete human CD2 domain 1 model shown in the same orientation as A. The CD2 binding site for CD58 has been represented: amino acids involved in CD58 binding are highlighted in red. Residues for those mutations in this study that produced no change in CD58 binding are shown in blue. The lysine-82 side chain is again picked out in yellow.

pletely abolished. This also applies to residue E36, conserved between human and rat CD2, in that its mutation to the oppositely charged residue R prevents the CD2/CD58 interaction. These results show for the first time the involve-

ment of  $\beta$  strand C in CD58 binding. The loss of rosetting when the positions of basic residues R48 and K49 in  $\beta$  strand C' are inverted is strong evidence that these amino acids are contact residues in the CD2/CD58 interaction.

Nonconservative mutations in the loops connecting  $\beta$  strands C and C' and  $\beta$  strands C' and C'', as well as residues in  $\beta$  strand C'', have no effect on the interaction of CD2 with CD58. This suggests that the side chains of these residues, although in the  $\beta$  sheet implicated in CD58 binding, are unlikely to be involved in contacts with the CD58 molecule.

The participation of the loop-connecting strands F and G in CD58 binding had been reported (30). Our results extend previous data by demonstrating that the mutation K91/T[86]+N92/R[87] blocks binding of CD58. From three mutations made on  $\beta$  strand F, only the change K82/N[77] disrupted CD58 binding. An identical disruptive mutant was described by Peterson and Seed (30). However, when residue K82 was substituted for an N in the context of mutant S84/Q, the mutant obtained, K82/N[77]+S84/Q, was capable of E-rosetting. These results indicate that the disruption of the CD2/CD58 interaction observed in mutant K82/N[77] is probably due to the attachment of a carbohydrate molecule to the new glycosylation site, NVS, generated by the mutation. This is also supported by the fact that the rat CD2 molecule is glycosylated at the equivalent position in strand F (21). A similar finding was recently reported for the interaction of TCR  $\beta$  chain and the Mls-1<sup>a</sup> superantigen (39). The presence of an N-linked carbohydrate in the region of V $\beta$ 8 that interacts with Mls-1<sup>a</sup> blocks its interaction with the superantigen.

In the recently published crystal structure of rat CD2, Jones et al. (21) described an interaction between two CD2 molecules in the crystal. This interaction is mediated by the CC' and FG loops and out-pointing residues on all the strands forming the CC'C''FG  $\beta$  sheet, except strand G. The exten-

sive crystal contact, although not necessarily representative of a physiological molecular interaction, involves some of the residues that the mutagenesis results have shown to be critical for CD58 binding.

Mutations that abolish E-rosetting with human erythrocytes also prevent rosetting with sheep erythrocytes, demonstrating that the same CD2 residues are involved in the interaction with both human and sheep CD58. This is despite the low level (47%) of conservation between the extracellular region of human CD58 (40) and the 74 amino acids known of the extracellular region of sheep CD58 (T. Hünig, personal communication). It has been proposed that CD2 and CD58 interact through the CC'C''FG  $\beta$  sheets of the opposed V-like domains, in a manner similar to the two Ig V domains of an Fab fragment (41). Determination of the complete sequence of sheep CD58 will allow the level of conservation of the CC'C''FG  $\beta$  sheet between sheep and human CD58 to be assessed and the possibility of an interaction through this surface to be examined.

The results presented in this report further demonstrate the versatility of Ig domains as recognition structures, with evidence accumulating that any surface of an IgSF domain may be exploited in both single or multiple adhesion interactions (42-47). For example, the CD8 molecule can use the CC'C''FG  $\beta$  sheet to form a dimer with another CD8 molecule and at the same time is able to interact with the MHC class I molecule through the CDR1 and CDR2 equivalent regions contained in the BC and C'C'' loops (42, 44). Future studies will reveal whether additional surfaces of the CD2 Ig-like domains are involved in interactions with CD59, CD48, or the CD3 complex.

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