

SIMILARITIES IN SEQUENCES AND CELLULAR EXPRESSION BETWEEN RAT CD2 AND CD4 ANTIGENS

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The MRC OX-34 mouse mAb was raised against rat T blasts and was found to label all T lymphocytes and most thymocytes but not B lymphocytes (1). The OX-34 antigen had an apparent mol wt of 50,000 (1), and it seemed possible that it was the rat equivalent of human CD2 (T11) antigen (2-4). However, OX-34 mAb labeled many more cells in rat spleen than could be accounted for by T lymphocytes (1), a property not described for human CD2 (2-4).

To further establish the nature of the OX-34 antigen, purification and molecular cloning was carried out, to yield the protein sequence. The expression of antigen in the thymus and spleen was also further investigated. The results show that OX-34 is rat CD2 and that the molecule is expressed in the T lymphoid lineage and on spleen macrophages. Comparison of the sequence with that of other antigens shows a highly significant relationship with CD4 antigen.

Materials and Methods

Animals. PVG/c and AO inbred rats were maintained in the SPF Unit of the MRC Cellular Immunology Unit. Sprague-Dawley rats were from Harlan Olac Limited, Bicester, United Kingdom.

Monoclonal Antibodies. MRC OX-34, IgG2a, anti-rat CD2 (1); MRC OX-35 and MRC OX-38, non-competing mAbs against rat CD4 (W3/25) (1); MRC OX-8, anti-rat CD8 (5); MRC OX-19, anti-rat CD5 (Ly-1) (6); MRC OX-12, anti-rat κ chain (7); ED3, anti-rat tissue macrophages (8) were provided by Dr. C. D. Dijkstra, Free University, Amsterdam, The Netherlands. MRC OX-21, anti-human C3b INA was used as a negative control.

Purification of OX-34 Antigen. Methods were as described previously (9). Purification was from deoxycholate extracts of rat thymocyte membranes that had been stored frozen after use in previous antigen purifications. Material from $\sim 2.5 \times 10^{12}$ thymocytes was passed through a 5-ml MRC OX-34 IgG affinity column (5 mg IgG/ml Sepharose 4B CL). The column was washed with deoxycholate buffer and then with pH 11.5 buffer without eluting the antigen. This was recovered by a second elution with 0.5 M propionic acid in the absence of detergent. Recovery of antigenic activity was measured by inhibition of an indirect radioactive binding assay (9).

Protein Chemistry. Standard methods were used: analysis by PAGE in SDS (1) on a Mighty Small gel apparatus (Hofer Scientific Instruments, San Francisco, CA); reduction and alkylation of antigen with ^{14}C -iodoacetic acid (10, 11); tryptic digestion; gel filtration of peptides on Biogel P30 in 0.1 M NH_4HCO_3 ; reverse-phase HPLC for peptide purification; amino acid analysis of protein and peptides (11). NH_2 -terminal and peptide

sequencing was on an Applied Biosystems, Inc., (Foster City, CA) gas phase sequencer or a Beckman Instrument, Inc., (Fullerton, CA) spinning cup sequencer with PTH amino acids detected by HPLC.

Nucleic Acid Chemistry. Oligonucleotide synthesis was by the phosphodiester method (12) and rat thymocyte cDNA libraries were prepared by the RNase H method (13). Cloning of cDNA was by standard methods (14) as used in previous studies (15). Sequencing was by the M13 method (16) and sequence analysis was via the programs of Staden (17). For statistical analysis of sequence the Align program was used (18). The scores were based on the Mutation Data Matrix (250 PAMS) with a bias and gap penalty of six. 150 random runs were used for all comparisons except the short sequences in Fig. 3c, for which the number was 100.

Analysis of Antigen Distribution. Labeling of lymphoid cell populations involved incubation with tissue culture supernatant containing MRC OX-34 mAb followed by purified fluorescein F(ab')₂ anti-mouse IgG antibody with analysis on a FACS flow cytometer (Becton Dickinson & Co., Mountain View, CA) (19). Rosette depletion of thymocytes with MRC OX-8, OX-35, and OX-38 was carried out using the method of Mason (19). Labeling of tissue cryostat sections was by the immunoperoxidase method (20) counterstained with Harris' hematoxylin. Acid phosphatase was detected by histochemistry as described previously (21) with modifications; 0.1 M sodium cacodylate buffer (pH 5) rather than Michaelis veronal acetate buffer, naphthol AS-BI phosphate was the substrate, incubation was for 40 min at 37°C.

Results

Purification of OX-34 Protein. OX-34 antigenic activity was substantially depleted from the detergent extract of rat thymocyte membranes after passage through the OX-34 affinity column, but the retained activity eluted very poorly (1% yield) with a pH 11.5 buffer in deoxycholate. Thus elution with 0.5 M propionic acid without detergent was tried and this gave a recovery of ~40% of the applied antigenic activity. The purified material is shown in Fig. 1A where one broad band is seen. By amino acid analysis, 1 mg of protein was recovered and this is a reasonable amount from 2.5×10^{12} cells given that 17,700 molecules of MRC OX-34 mAb are bound per Sprague-Dawley thymocyte (22) and that the antigen yield from cells to detergent extract is usually ~70% (9).

The material in Fig. 1A shows a diffuse band with a leading edge of 42,000 apparent mol wt. This is less than the mol wt of 50,000 determined for OX-34 labeled at the cell surface with ¹²⁵I, and it seems likely that the material in Fig. 1A is partially degraded. The amino acid analysis is shown in Fig. 1B, and from this and the protein sequence below it seems likely that much of the cytoplasmic domain has been lost. We draw this conclusion since proline is concentrated in the cytoplasmic tail (Fig. 3B) and the percentage for Pro from the sequence is 10%, while the value by analysis from the purified protein is only 3.9% (Fig. 1B).

Protein and Peptide Sequence. ~20 nmol OX-34 protein was reduced and alkylated and 18 nmol was digested with trypsin for peptide purification while 2 nmol was subjected to NH₂-terminal sequencing (Fig. 1A). Peptides were purified by gel filtration and reverse-phase HPLC and were selected for sequencing on the basis of their amino acid analysis. Peptide 2 was chosen as a candidate for oligonucleotide probe sites while peptide 1 was sequenced because of the presence of glucosamine in the analysis (see legend to Fig. 1). Peptide 3 was sequenced to confirm the predicted sequence after the transmembrane segment. All the

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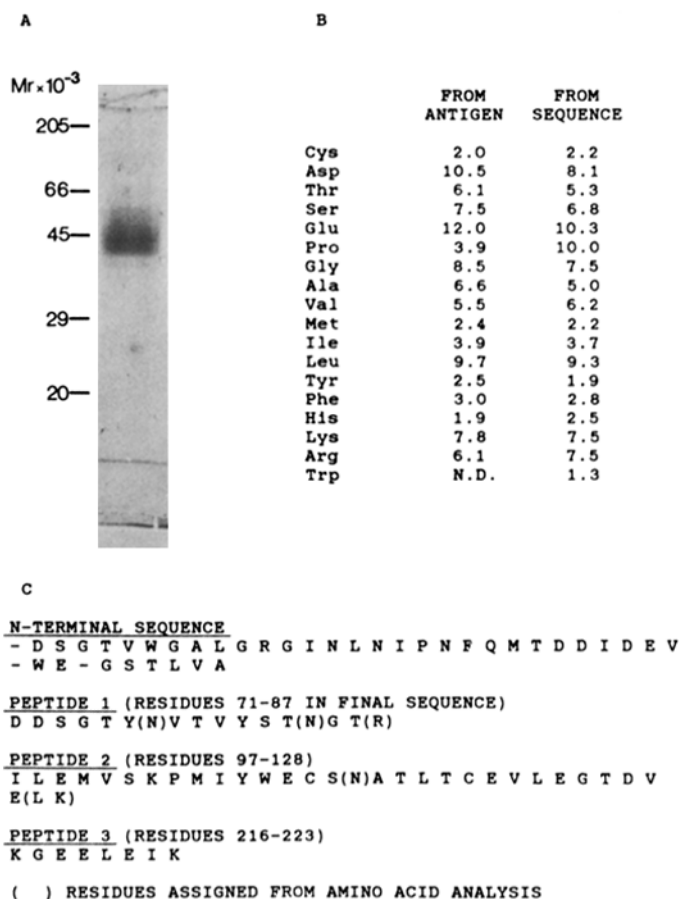


FIGURE 1. Analysis of OX-34 antigen and peptide sequences. (A) 2.5 μ g of purified OX-34 antigen electrophoresed on a 12% polyacrylamide gel in SDS and stained with Coomassie blue. (B) Amino acid analysis (residues per 100 amino acids) derived from duplicate analyses of 10 μ g of OX-34 antigen after oxidation with periodic acid and hydrolysis for 24 h at 110°C with 5.7 N HCl and 0.01 M phenol. Destruction of Ser and Thr was not corrected for, and Trp was not detected. The analysis from purified antigen is compared with that calculated from the sequence in Fig. 3B. (C) Protein sequences. The NH₂-terminal sequence was determined from 2 nmol of reduced and alkylated antigen in the gas phase sequencer. At the first position many anomalous peaks were seen when the PTH amino acid derivatives were run on HPLC and no residue could be assigned (the same result was obtained on a second run with unreduced antigen). After that, one residue was clearly assigned at each position through to Ala at residue 40, with the exception of positions 31 and 34 where no residues were detected (these were later found to be Arg residues from the cDNA sequence). The yield at Asp 2 was 350 pmol and the repetitive yield was 97%. The peptide sequences were determined on the Beckman Instruments, Inc., spinning cup sequencer from 4, 6, and 7 nmol of peptides 1, 2, and 3, respectively. The initial and repetitive yields were: peptide 1, 1.6 nmol, 95%; peptide 2, 3.1 nmol, 90%; peptide 3, 5 nmol, 92%. All the assignments were unambiguous with the exception of the positions shown in brackets, at which no residue was detected. The bracketed residues were assigned from amino acid compositions as follows, with all residues not given showing <0.2 mol/mol peptide. (*Peptide 1*) Asp (3.7), Thr (3.4), Ser (2), Gly (1.7), Val (2.2), Tyr (1.4), Arg (1), glucosamine (5.4). (*Peptide 2*) Cys (2), Asp (2), Thr (2.8), Ser (2.2), Glu (5.2), Pro (0.9), Gly (1.2), Ala (1.5), Val (2.8), Met (1.5), Ile (1.6), Leu (3.5), Phe (0.9), Lys (2), glucosamine (3.3). (*Peptide 3*) Ser (0.5), Glu (2.9), Gly (1.1), Ile (0.9), Leu (1), Lys (1). In peptides 1 and 2 glucosamine was detected in good yield even though peptide hydrolysis conditions were used and the amounts indicate the presence of 2 and 1 N-linked carbohydrate structures in each case. These are assigned to Asn residues at positions 7 and 14 in peptide 1 and residue 16 in peptide 2. Glycosylated residues are not detected in the sequencer run.

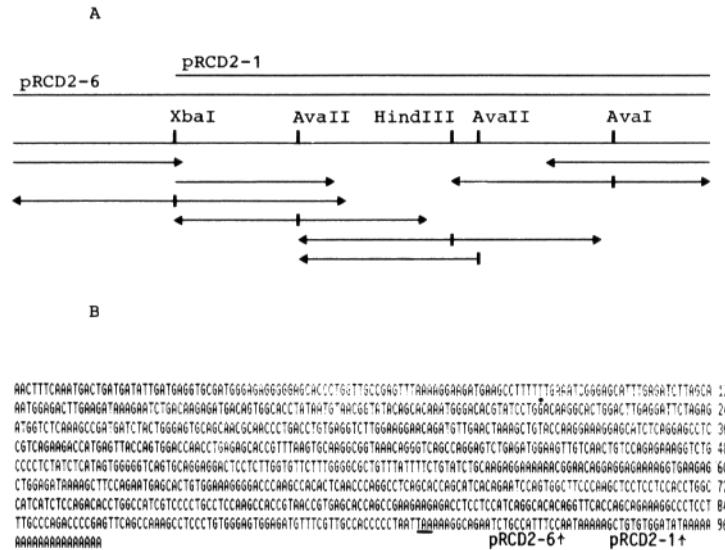


FIGURE 2. Rat CD2 cDNA clones and nucleotide sequence. (A) restriction enzyme sites used in sequencing are shown and the *arrows* indicate the sequences that were determined. (B) the combined nucleotide sequence is shown with the stop codon underlined. The *dot* above residue 211 indicates a position that differed in the two clones (see text). The *arrows* indicate positions where the poly(A) tails started in each clone.

peptides gave unambiguous sequences, which agreed with the amino acid compositions given in the legend to Fig. 1. In the NH₂-terminal sequence the first residue was not determined due to multiple peaks at this position. At other positions residues could be clearly assigned.

cDNA Cloning. Two anti-sense-mixed oligonucleotide probes were synthesized on the basis of the sequence of peptide 2. The peptide sequences used were: KPMIYW to give 17 nucleotides containing a total of 48 sequences, and MIYWEC to give 17 for 12. In primary screening of a rat thymocyte cDNA library the lower redundancy probe was used first and possible positives were checked by a second screen with the other probe. From a library with a complexity of 60,000, eight colonies were picked and one of these, pRCD2-1, was correct. This cDNA clone was not full length and thus other libraries were screened with pRCD2-1 cDNA labeled by nick translation. The positive clones were rescreened with either of the oligonucleotides above, plus another containing 14 nucleotides at a redundancy of 8, which was predicted from the sequence NFQMT beginning 20 residues from the NH₂-terminus (Fig. 1C). In this way the cDNA clone pRCD2-6 was isolated from a rat lymph node cDNA library; the relationship of this to pRCD2-1 is shown in Fig. 2. The sequence predicted from pRCD2-6 was still not full length, but as expected overlapped with the NH₂-terminal protein sequence (see below).

Nucleotide and Predicted Protein Sequence. The nucleotide sequencing strategy is shown in Fig. 2A and all bases were determined by sequencing in both directions such that each position was determined, on average, 5.5 times. An open reading frame was found in both pRCD2-1 and pRCD2-6 and the only difference between the two clones in this region was a guanine at nucleotide 211

in pRCD2-1 vs. adenine in pRCD2-6 (Fig. 2). This leads to a coding difference of Asp vs. Asn at amino acid 90 (Fig. 3). Both libraries were constructed from AO strain rats but not from the same colony, and thus the difference could be due to a polymorphism or alternatively to a sequence change occurring during cDNA synthesis.

The two clones had poly(A) tails in different positions after extremely short 3' noncoding regions (Fig. 2B). The poly(A) tails started at 47 and 26 nucleotides after the last coding nucleotide for pRCD2-1 and pRCD2-6, respectively. The poly(A) addition signals are presumably the sequences AATAAA and ATTAAA starting at 26 and -2 nucleotides after the last coding nucleotide (23). Neither clone contained 5' sequence extending to a predicted leader sequence, but the protein sequence predicted from Fig. 2 overlaps with the NH₂-terminal protein sequence (Fig. 1) to yield the full protein sequence, except for the first residue as shown in Fig. 3.

The peptide and NH₂-terminal sequence given in Fig. 1 fitted exactly with the predicted protein sequence from the cDNA shown in Fig. 3B and established the sequence as correct until amino acid 223. The predicted protein sequence shows a very hydrophobic stretch of amino acids at residues 178-206, and this is likely to span the membrane. The sequence NH₂-terminal to the hydrophobic piece is likely to be extracellular because it includes four *N*-linked carbohydrate sites (Fig. 3B), three of which are known to be glycosylated from the peptide data (Fig. 1). A glycopeptide accounting for the fourth site has not yet been isolated. The cytoplasmic domain is likely to start with the cluster of basic residues after the hydrophobic sequence and is unusual, with Pro accounting for 26 of 116 positions.

Comparison with other Sequences. Comparison of the OX-34 sequence with that for human CD2 as determined by Sewell *et al.* (24) established that OX-34 is rat CD2. Overall the sequences were 56% identical, and a notable feature was the finding that a block of 24 residues in the cytoplasmic domain of each sequence was identical (sequence 287-310 in rat CD2). Apart from the biological interest of this, these identities prove that the putative cytoplasmic domain is correct in spite of the fact that peptides corresponding to this region were not isolated. In the pure antigen it seems likely that this part of the sequence has been lost by proteolysis that may have occurred during successive freezing and thawing of the detergent extracts from which the antigen was isolated, even though inhibitors of proteolysis were added.

Comparison of the rat CD2 sequence with those of other cell surface antigens showed highly significant matches with rat and human CD4 antigen sequences. The relationships are shown schematically in Fig. 3A and as an alignment between rat CD2 and rat CD4 in Fig. 3B. The significance of the alignment was tested using the Align program, which scores the best match for the test sequences and then compares this score with a mean maximal score from a chosen number of comparisons using sequences compiled from the test sequences after randomization. The score for the real sequences is given as SD units away from the mean random score, and the significance of these can be roughly judged from relationships between the probability of a chance result and SD scores as follows: probability 10^{-2} , SD score 2.3; 10^{-3} , 3.1; 10^{-5} , 4.3; 10^{-7} , 5.2; 10^{-9} , 6.0 (reference

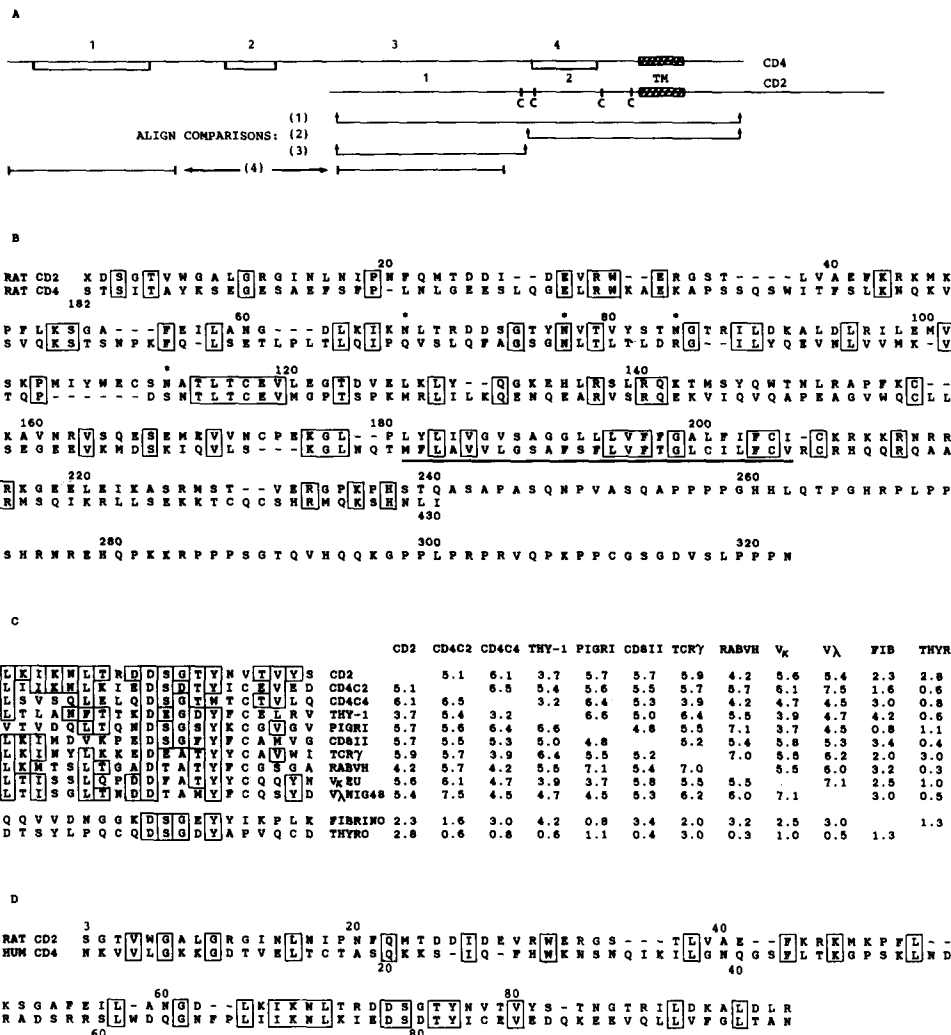


FIGURE 3. Sequence of CD2 and comparison with CD4. (A) Shows schematically the alignments of CD4 and CD2 sequences with regions that were compared in statistical analysis using the Align program. The positions of disulphide bonds are based on data from mouse CD4 (33). (B) Rat CD2 aligned with the second half of rat CD4 (25) with the CD2 sequence continuing on after the last CD4 residue (182-430). The alignments are as given by the Align program and identical residues are boxed. Dashes indicate gaps inserted to maximize the similarities. Asterisks indicate N-linked glycosylation sites in rat CD2 and the line under the sequence at CD2 residues 181-205 indicates stretches of hydrophobic sequence common to both molecules. The unknown residue at position one of CD2 is indicated as X. (C) Alignments of CD2 residues 63-82 with V domains and V-related sequences around the conserved COOH-terminal Cys of Ig or Ig-related domains plus two sequences selected as controls on the basis that they had the DSGXY pattern that initially drew attention to this section of CD2. The sequences are as follows with the NBRF data base number (reference 26) or literature reference given in parentheses. CD4C2 and CD4C4, human CD4 around Cys 86 and 161, respectively; (34), Thy-1, mouse Thy-1.1 (TDMS); PIGRI, rabbit poly(Ig) receptor domain I [QRRBG]; CD8II, 37-kD chain rat CD8 (35); TCRY, T receptor V γ [RWMSVB]; RABV_H, rabbit Ig V_H [HVRBP3]; V_K EU, human Ig V_K myeloma EU [KIHUEV]; V λ NIG48, human Ig V λ myeloma NIG48 [L6HU48]; FIBRINO, fibrinogen γ chain precursor [FGLMGS]; THYRO, thyroglobulin precursor [UIHU]. (D) Alignment of rat CD2 domain 1 with human CD4 domain 1 as given by the Align program. Boxes show identical residues and gaps are shown by dashes.

18). For comparison 1 as shown in Fig. 3A, the score for rat CD2 vs. rat CD4 and human CD4, respectively, was 7.7 SD and 6.8 SD; for comparison 2 the values were 7.9 SD and 6.9 SD; and for comparison 3 they were, respectively, 2.1 SD and 2.3 SD. From this we conclude that there is a very strong case for a relationship between CD2 and CD4 in the membrane proximal domain and transmembrane sequence, but that the match of CD2 domain 1 and CD4 domain 3 is only weakly statistically supported even though some sequence similarities are evident by eye.

Elsewhere it has been argued that domain 3 of CD4 is Ig-related even though it lacks a disulphide bond (25), and domain 1 of CD2 is also of interest in this regard. Within CD2 domain 1, the sequence at residues 63–82 is highly similar to sequences of Ig V- or V-like domains around the COOH-terminal Cys of the conserved disulphide bond (Fig. 3C), even though the CD2 sequence has no Cys. The similarity is such that when the NBRF data base (26) was searched with this sequence using the FASTP program (27), 16 of the top 20 matches were with Ig or Tcr V domains or other V-related sequences (data not shown). The significance of the match can also be judged by the matrix of Align scores for the short sequences in Fig. 3C. The scores for the CD2 sequence vs. the V- or V-related sequences are extremely good when related to the scores obtained within the Ig-related sequences or to the scores for two control sequences. The controls were selected by searching the NBRF data base for sequences with the peptide DSGXY, which is a major part of the V-like pattern that initially indicated that the CD2 sequence residues 63–82 may be Ig-related. In addition to this, the whole of CD2 domain 1 can be aligned with domain 1 of CD4 (which is clearly Ig-related) to show a significant overall relationship (Fig. 3D), including the short patch of sequence shown in Fig. 3C. The Align score for the alignment shown in Fig. 3D (human CD4) is 3.2 SD, while for rat CD4 the score was 1.7 SD. In the same region human CD2 gave scores of 3.4 and 5 for rat and human CD4 respectively. Taken together, these scores provide good evidence that CD2 domain 1 is related to domain 1 of CD4 and also to Ig-V domain sequences, even though it lacks the conserved Cys residues of typical Ig-like domains.

Cellular Expression of OX-34 Antigen. In humans, CD2 antigen has been reported (28) to be expressed only on thymocytes, T lymphocytes, and NK cells, and in the T lineage the antigen is expressed at an early stage. Expression of OX-34 in the rat T cell lineage was examined and the results are shown in Fig. 4. 60% of lymph node cells were labeled (Fig. 4A) and overlap experiments with other markers established that the OX-34⁺ cells included all T cells (1). Most thymocytes were positive, albeit with weak labeling for some cells (Fig. 4B). Only 2–4% of bone marrow cells showed labeling above control values (Fig. 4C). Peripheral T cells stained more intensely than the majority of thymocytes; however, CD4⁻, CD8⁻ thymocytes, which include the rat thymocytes that are active in thymopoiesis (29), are split into two sets by OX-34, with 59% of the cells showing clear labeling of a similar intensity to peripheral T cells. The rest were unlabeled or very weakly labeled (Fig. 4D).

In preliminary studies (1), MRC OX-34 was found to label many non-T cells in spleen sections and this is shown in Fig. 5a. The position of T cells in the spleen was established by labeling with OX-19 mAb (anti-CD5 [Ly-1]) (Fig. 5c).

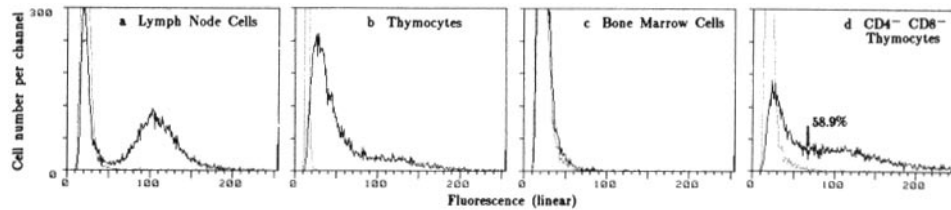


FIGURE 4. Labeling of lymphoid cells with MRC OX-34 mAb. Cells were labeled with OX-34 mAb in the form of tissue culture supernatant, followed by fluorescein F(ab')₂ anti-mouse IgG, with both steps at 4°C. Fluorescence histograms were obtained on a Becton Dickinson & Co. FACS II with 10⁶ cells analyzed for each profile and with cell number shown on a linear scale. CD4⁻CD8⁻ thymocytes were prepared by rosette depletion. Labeling with MRC OX-21 mAb is shown as a negative control for each cell type (*dotted line*).

The non-T OX-34⁺ cells are prominently seen in the marginal sinus, the marginal zone, and the red pulp. The pattern of non-T cells was very similar to that shown by the ED3 macrophage marker (8) (Fig. 5*b*). To establish whether these cells were macrophages, sections were double labeled for the acid phosphatase macrophage marker and OX-34 antigen. Fig. 5, *e-g* show, respectively, OX-34 labeling, acid phosphatase labeling, and double labeling. When the double-labeled sections were studied under the microscope there was no doubt that the large cells in the marginal zone were labeled by OX-34 mAb and were acid phosphatase-positive. This was also likely to be the case for cells in the red pulp, but the interpretation was more difficult since individual cells were not clearly defined.

In previous studies (1) it was found that OX-34 mAb did not label peritoneal macrophages that were CD4⁺. In this study, liver macrophages were also found to be OX-34⁻ in comparison with clear-cut labeling with anti-CD4 mAbs (Fig. 5, *h* and *i*).

It could be argued that labeling of spleen macrophages with OX-34 is due to an irrelevant crossreaction, and to check this, new anti-rat CD2 mAbs are being raised after immunization with pure OX-34 antigen. One new mAb that competes with OX-34 in binding to cells has been isolated thus far and this gives the same labeling pattern in the spleen as OX-34 mAb.

Discussion

The OX-34 antigen has been purified and cDNA clones have been obtained to yield the sequence of the fully processed molecule, with the exception of residue 1. Comparison with the sequence of human CD2 (T11) antigen (24) showed that OX-34 is rat CD2 (data not shown). The sequence has a number of interesting features and is notable for a cytoplasmic domain of 116 residues, which is considerably larger than that of many other molecules of the T lymphocyte surface. It was particularly interesting that a segment of 24 residues in the cytoplasmic domain was identical between rat and human sequences and thus may represent a binding domain for a cytoplasmic enzyme or structural element.

The rat CD2 sequence showed clear-cut matches with CD4, and the significance of these similarities can be summed up by the fact that the Align scores

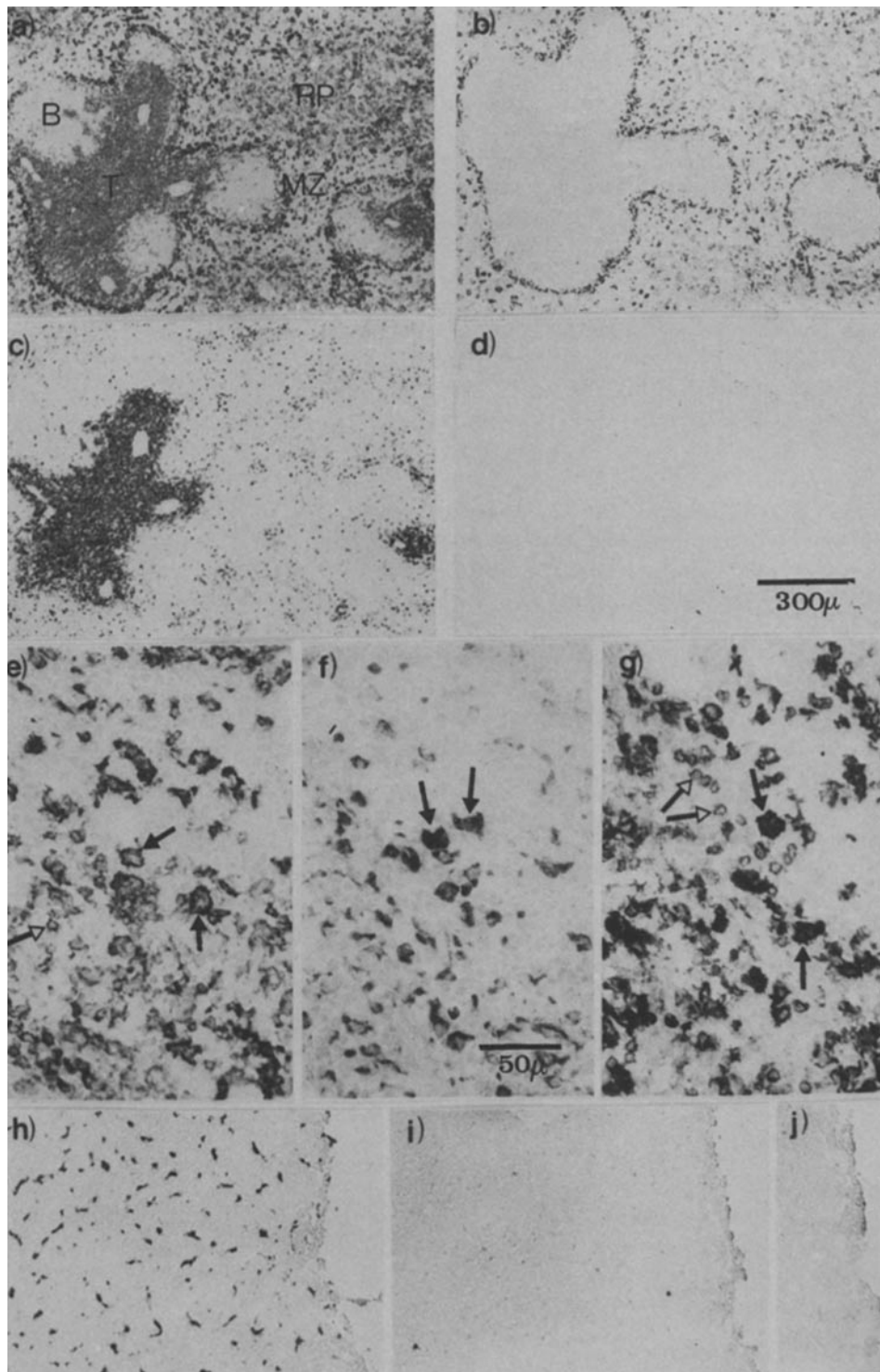


FIGURE 5. Distribution of OX-34 antigen in spleen and liver. Frozen sections of spleen were stained using the immunoperoxidase technique with mAbs: (a) MRC OX-34, (b) ED3, (c) MRC OX-19, (d) MRC OX-21. T, T cell area, B, B cell area, MZ, marginal zone, RP, red pulp. (e-g) High power micrographs of the marginal zone and red pulp are shown. (e) MRC OX-34 immunoperoxidase, (f) acid phosphatase histochemistry, (g) MRC OX-34 plus acid phosphatase. Open arrows, T cells. Closed arrows, large cells identified as macrophages. Frozen sections of liver were stained using the immunoperoxidase technique with mAbs (h) MRC OX-35 and MRC OX-38, (i) MRC OX-34, and (j) MRC OX-21.

for the segment of ~100 residues in Fig. 3A (comparison 2) are at about the same level as is obtained for various Tcr vs. Ig V domain comparisons.

Is CD2 a member of the Ig superfamily? The CD2 membrane proximal region closely matches CD4 domain 4, with the Cys residues at 117 and 157 being in positions equivalent to the Cys residues that form the third disulphide bond in CD4. In an analysis of CD4 sequences (25) it has been argued that domains 1-4 as shown in Fig. 3A were all originally derived from Ig-related domains, and if this is accepted then CD2 should be seen as another of the Ig-related molecules. In the current analysis, evidence is presented for a relationship between domains 1 of CD2 and CD4, and this provides a further link to the Ig-related sequences since CD4 domain 1 has strong similarities to Ig V domains. Domain 1 of CD2 is also identified as a second sequence, in addition to domain 3 in CD4, that may be Ig-related and possibly folded like a V domain without the presence of the conserved disulphide bond.

Similarities between CD2 and CD4 were seen in cellular expression as well as structure. CD2 appears to be on all rat thymocytes and T lymphocytes with the exception of ~40% of CD4⁻,CD8⁻ thymocytes, and this is consistent with the finding that CD2 is expressed early in the human T lymphocyte lineage. In the rat it will be interesting to see whether or not thymopoiesis can be given by the two subsets of CD4⁻,CD8⁻ cells that are marked by OX-34 mAb (Fig. 4). The CD4 antigen is expressed a little later than CD2 in the T cell lineage and is ultimately found on a clear subfraction of peripheral T cells that are all CD2⁺. Both antigens are also found on rat macrophages (1, 20); however, in this case, the CD4 antigen shows a broader macrophage distribution while expression of CD2 is clearly on a macrophage subset. The macrophage CD2 expression remains to be fully investigated but the antigen seems to be present on most spleen macrophages but absent from peritoneal and liver macrophages. The pattern of CD2⁺ macrophages in the marginal zone and the marginal sinus was particularly striking.

In humans the CD2 antigen has been implicated in two functional aspects. Mixtures of anti-CD2 mAbs are mitogenic for T cells (30), and it has been recently suggested (31) that CD2 is a receptor for a T cell growth factor that plays an important role at an early stage in the T lymphocyte lineage. Other work (32) provides evidence for a role for CD2 in adhesion reactions between lymphocytes and other cells. The data in the present paper do not strongly relate to functional aspects since the OX-34 mAb has no functional effects on proliferative T cell responses. However, the expression of CD2 on macrophages as well as T cells may be more compatible with the idea that the molecule plays a role in cell-cell interactions rather than as a growth factor receptor. Clearly, more data is required and multiple roles for cell surface molecules cannot be excluded.

Summary

The MRC OX-34 antigen of rat T lymphocytes was purified and peptide sequences were obtained. Oligonucleotide probes were synthesized and cDNA clones coding for the antigen were isolated and sequenced to yield a predicted protein sequence for the molecule that fitted the peptide data. Comparison of

this sequence with that for human CD2 determined by Sewell et al. (24) showed that OX-34 is rat CD2. The primary structure of the molecule was notable for a moderately large cytoplasmic domain of unusual sequence and also for its highly significant relationship to CD4 antigen in the membrane proximal extracellular region and the transmembrane sequence. A relationship to the Ig superfamily can be argued for the two extra cellular domains of CD2, even though neither fits the standard pattern for Ig-related domains.

Within the T lymphocyte lineage, rat CD2 seemed to be present on all stages with the exception of ~50% of the thymic CD4⁻,CD8⁻ cells. In addition, the antigen was prominent on most macrophages in the spleen but not found on peritoneal or liver macrophages. CD4 antigen is also expressed on T lymphocytes and macrophages, and thus CD2 and CD4 appear similar in their cellular expression as well as structural characteristics.

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