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An antiserum raised against the recombinant cytoplasmic tail of the human CD43 glycoprotein identifies CD43 in many mammalian species

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SUMMARY

Leukosialin or CD43 is a heavily O-glycosylated transmembrane protein expressed on all cells of the haematopoietic cell lineage with the exception of red blood cells and mature B cells. This antigen has been identified in human, mouse and rat with monoclonal antibodies. Although orthologues of many human and rodent leucocyte cell surface antigens have been described in recent years, CD43, despite its abundance on human and rodent cells, remained uncharacterized in other vertebrate species. The comparison of CD43 amino acid sequences from human, mouse and rat indicated a high level of homology in the cytoplasmic domain. A serum, (p.aCD43cp) raised against the recombinant cytoplasmic tail of the human CD43, was shown not only to recognize human CD43, but it bound to putative CD43 orthologues in many mammalian species. CD43 was found to be expressed in the same leucocyte subpopulations and circumstantial evidence suggested that CD43 is also regulated similarly during leucocyte ontogeny in all species investigated. As $CD43^+$ cells were readily observed in fixed tissues, the p.aCD43cp serum may be used as a reliable reagent for the verification of the haematopoietic origin of infiltrations and, used together with other reagents, for the serological characterization of normal and pathological lymphoid tissues and lymphoid infiltrations in experimental work and in animal disease.

INTRODUCTION

Almost two decades ago lectin-binding experiments identified a sialated glycoprotein in human and rodents. The diverse designations (sialophorin, leukosialin, W3/13 antigen) of this antigen were unified as CD43.[1–3]

The biochemical properties of CD43 are unique. It consists of about 400 amino acid residues in humans and rodents the sequence of which shows no obvious homology to any other known protein.[4–6] The extracellular domain contains a very high proportion of serine and threonine residues most of which are hypothesized to undergo O-glycosylation.[7] The glycosylation is dependent on the type and the activation state of the cells resulting in a lot of different activation and cell type-specific forms[8–10] and is known to change in pathological conditions.[11,12] All isoforms are glycoforms that differ only in their composition of carbohydrate moieties and are not a result of alternative splicing. In addition, as glycosylation is not a tightly regulated process, even molecules from the same cells may be differently glycosylated resulting in microheterogeneity of the CD43 glycoproteins.[7]

The expression of this protein is restricted to haematopoetic cells in non-pathological conditions. It is present in all major white blood cell lineages, but it is transcriptionally down-regulated in mature B cells and erythrocytes.[13] It can also be shed proteolytically from some haematopoetic cells upon cell activation.[14,15] In pathological conditions CD43 may be expressed in non-haematopoetic tissues, like colon carcinoma cells.[16]

Despite exhaustive functional studies the role of this glycoprotein is still poorly understood. Treatment of white blood cells with CD43-specific monoclonal antibodies are accompanied by characteristic changes. The overall adhesiveness of the cells increases, the cells bind viruses [(for example human immunodeficiency virus (HIV)], matrix proteins (fibronectin), other cells (heterotypic aggregation) and each other (homotypic aggregation) more effectively. The adhesion is not mediated through CD43 as initially thought, but through different adhesion structures like integrins.[17,18] There are only occasional descriptions of monoclonal antibodies that in themselves induce activation of cells, but most of CD43 monoclonal antibodies (mAbs) are potent co-stimulators used in combination with other stimulatory agents.[17,19] The experiments detailed above suggested that CD43 could be a co-receptor which upon ligand binding transduces signals to the cells. The putative ligand has been intensively sought for. Intracellular adhesion molecule 1 (CD54), galectin-1 and major histocompatibility complex-I

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(MHC-I) have been proposed as candidates, but these results still await confirmation.[20–22]

Leucocyte subsets from the CD43$^{-/-}$ mouse generated by gene targeting show phenotypes that are similar to cells treated with CD43 monoclonal antibodies. That is, they possess an increased adhesiveness and susceptibility to stimulation.[23] Based on the current evidence, CD43 may play a role on one hand in the cell-to-cell adhesion as a negative regulator, and on the other hand in signal transduction. Although the protein is not essential for the development of any particular subsets of murine lymphocytes, immune responses against some pathogens are drastically impaired as judged by the phenotype of CD43$^{-/-}$ mice, providing evidence that CD43 has an important role in the mammalian immune system.[23]

Leukosialin or CD43 has been characterized by monoclonal antibodies in human, mouse and rat. Although CD43 is an abundant and immunodominant protein in humans and rodents, international workshops organized for the characterization of leucocyte cell surface antigens by monoclonal antibodies raised against extracellular epitopes of leucocytes failed to identify the ruminant, swine, equine, feline and canine CD43 orthologue.

Several monoclonal antibodies raised against leucocyte cell surface antigens of a particular species cross-reacted with similar leucocyte antigens of closely or distantly related species.[24–26] However, in the case of the CD43, no monoclonal antibody is known to cross-react between species. Comparison of human, murine and rat CD43 amino acid sequence reveals a very low homology in the extracellular region. Any immunoreagent against extracellular epitopes of CD43 is therefore unlikely to show cross-reactivity between species. In contrast, the intracellular tail of the glycoprotein is remarkably conserved. In this study we investigated whether an immunoreagent that recognizes the cytoplasmic tail of the human CD43 allows the detection of this high degree of sequence similarity at the serological level in various animal species.

MATERIALS AND METHODS

Generation of the pGEX-KN-CD43cp construct

The region encoding the cytoplasmic tail of human CD43 was amplified by polymerase chain reaction (PCR) using 10pg pHF1 plasmid,[27] a genomic clone encoding both exons of the CD43 protein as a template and CGGGGTACCAAGGAGGTCCGGATG*GCCGGCGGCAGAAGCGG* and CGGGAATTCAAGCTT*TTAAGGGGCAGCCCCGTC* oligonucleotides as 5′ and 3′ primers, respectively. Hybridizing sequence stretches are shown in italic. PCR was carried out in a reaction buffer containing 10 mM Tris/HCl (pH = 8·8) (Cetus Corp., Emeryville, CA), 50 mM KCl (Cetus), 3·5 mM $MgCl_2$ (Merck, Darmstadt, Germany), 200 μM each dNTP(Cetus) and 2·5 U *Taq*I (Cetus). The template was denatured for 10 min. Cycling parameters were as follows: denaturation at 94° for 90 seconds, annealing at 55° for 60 seconds, and extension at 72° for 180 seconds.

The PCR product was cleaved on one end with *EcoRI* (NEB, Beverly, MA) and on the other end with *Sac*II (NEB) at cleaving sites that were introduced by the non-hybridizing stretches of the primers and cloned into the pBC-SK$^+$ (Stratagene, La Jolla, CA) vector. DNA from insert containing white colonies were restriction mapped and sequenced on both strands using T3 and T7 primers to ensure the fidelity of the amplified product.

The plasmid for the expression of the recombinant protein in the form of a glutathione-S-transferase (GST) fusion protein was constructed by swapping an irrelevant insert of the pTS1.14/5 plasmid (unpublished result) to the *Sac*II–EcoRI fragment encoding the cytoplasmic tail of the CD43 protein. The pTS1.14/5 is similar to the pGEX-KN-PTP$_{U323}$ expression construct[28] with a difference of an introduced *Sac*II recognition site (CCGGCC) that codes for proline and arginine amino acids of the thrombin cleavage site.

Purification of the CD43cp recombinant protein

Bacteria were grown to $OD_{600} = 0{\cdot}6$, induced in the presence of protease inhibitors [0·2 mM phenylmethylsulfonyl fluoride (PMSF) (Sigma, St Louis, MO) added every 20 min and 25 μM para-nitrophenyl-p′-guanidino-bensoate (Sigma)] for 1·5 hr at 37° by the addition of 0·1 mM isopropyl β-thiogalactopyranoside (IPTG) (Sigma), washed twice in phosphate-buffered saline (PBS) and finally disrupted by French press in the presence of 1 mM PMSF, 10–30 μg/ml aprotonine (Sigma), 25 μM para-nitrophenyl-p′-guanidino-bensoate (Sigma) and 2–10 mM EDTA. The resulting slurry was separated into soluble and insoluble fractions by centrifugation. The bulk of the recombinant protein was shown to be located in the soluble fraction. The lysate was pre-cleared by filtration and flowing through a bovine serum albumin (BSA) -coupled Sepharose CL-4B column. The GST-CD43cp fusion protein was purified by a Glutathion Sepharose 4B affinity chromatography (Pharmacia, Uppsala, Sweden) as recommended by the manufacturer. The CD43cp fusion partner was cleaved off to the supernatant using thrombin (Pharmacia) overnight digestion at room temperature.

Production and affinity purification of the CD43cp serum

Rabbits were immunized with the CD43cp protein using standard procedures. The serum obtained after the third round of immunization was affinity purified in two steps. First, the antibodies reacting with the GST or other proteins of the *Escherichia coli* were depleted using a Sepharose CL-4B column (Pharmacia) coupled with the lysate of *E. coli* overproducing GST. The eluted antibody fraction (p.a*E. coli*-GST) was used as an irrelevant control in subsequent experiments. In the second step the antibody fraction specific for the CD43cp protein was purified in a Sepharose CL-4B column (Pharmacia) coupled with the lysate of GST-CD43cp overproducing *E. coli*. The specificity and the purity of the antibody fractions was confirmed using enzyme-linked immunosorbent assay (ELISA) and sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE).

Antibodies

Monoclonal antibodies used in our studies were OKT3 (Ortho Diagnostic Systems, San Diego, CA), MA5E,[29] HD37 (DAKO, Glostrup, Denmark) and T2/53[30] recognizing extracellular epitopes of CD3ε, CD14, CD19 and CD43, respectively. Isotype-matched irrelevant monoclonal antibodies served as negative controls. A pan-T-cell antiserum, CD3 T cell (DAKO) referred to as p.aCD3ε(177–190) that was raised against a peptide corresponding to an intracellular stretch of

the human CD3ε antigen, was used to detect T lymphocytes in various vertebrate animals.[31,32]

Cells and tissues

P3H1 and Jurkat cell lines were grown in RPMI-1640 medium supplemented with 5% fetal calf serum (FCS).

Mononuclear cells from heparinized peripheral human blood (human PBM cells) were separated by Ficoll-iodamide gradient density centrifugation. White blood cells from heparinized mammalian blood were obtained after two rounds of red-blood-cell lysis with distilled water.

Various lymphoid and non-lymphoid tissues from cattle, swine, chicken, turkey, duck and goose were obtained from the local slaughter house. Various organs from carp (Cyprinus carpio) and edible frog (Rana esculenta) were dissected by us.

Formalin-fixed paraffin-embedded tissue samples from different pathological cases (1982–92) were obtained from the archive collection of the Central Veterinary Institute (Budapest, Hungary). When possible each disease was represented by at least three independent cases.

Cell lysis and immunoprecipitation

Cells were lysed in a lysis buffer containing 10 mM Tris pH = 8·0, 150 mM NaCl, 2 mM EGTA, 2 mM $MgCl_2$, 1% NonidetP-40 and a cocktail of protease inhibitors, for 10 min on ice. Lymphoid and non-lymphoid tissues were disrupted in a Dounce homogenizer (Fisher, Düsseldorf, Germany) in the same buffer. Immunoprecipitation from the postnuclear supernatant was carried out for 2 hr at 4° using affinity-purified antisera coupled directly, or monoclonal antibodies coupled via a rabbit anti-mouse immunoglobulin (DAKO) to ProteinA–Sepharose CL4B (Pharmacia). The immunoprecipitates were washed three times in lysis buffer.

SDS–PAGE and immunoblotting

Protein-containing fractions were boiled in reducing Laemmli's sample buffer and analysed by SDS–PAGE. After the separation proteins were transferred to polyvinylidene-difluoride (PVDF) membranes (Millipore, Bedford, MA). The filters were blocked in Tris buffered saline (TBS) containing 3% non-fat milk powder and 0·1% Tween 20 (Sigma) for 1–2 hr, incubated with the first antibodies in a final concentration of 5–30 μg/ml and washed extensively in TBS containing 0·1% Tween 20. Then, the membranes were incubated with Horse Radish Peroxidase-conjugated ProteinA (Sigma) or rabbit anti-mouse immunoglobulin (DAKO), as recommended by the manufacturer, and washed thoroughly. Immunoreactive proteins were visualized by enhanced chemiluminesecence (ECL, Amersham, Aylesbury, UK) as recommended by the manufacturer.

Indirect immunofluorescence

$2–5 \times 10^5$ cells were washed once in PBS containing 1% BSA (Sigma) and incubated with 5–10 μg/ml of different monoclonal antibodies recognizing extracellular epitopes of cell surface proteins for 45 min on ice. The cells were washed three times and fluorescein isothyocyanate (FITC)-conjugated rabbit anti-mouse immunoglobulins (DAKO) were added according to the manufacturer's instructions.

Cells were permeablized for 10 min on ice using 25–50 μg/ml water-soluble digitonin (Sigma) dissolved in PBS containing 2 mM EGTA (Sigma), 2 mM $MgCl_2$ (Merck) and 1% BSA. The permeability of the cells was then confirmed by the abrogation of Trypan Blue exclusion. The procedure was shown not to affect the labelling of the extracellular epitopes. The cells were washed with PBS containing 1% BSA to remove the permeabilizing agent.

The permeabilized cells were incubated with the biotinylated affinity-purified antisera for 45 min on ice. Fc-receptors were blocked using normal sera of the animals from which the cells were derived. Human PBM cells were found to bind rabbit immunoglobulins so strongly that normal rabbit antiserum was also added. Binding of biotinylated immunoglobulins to biotin-binding intracellular proteins were minimized by addition of 10 μg/ml d-biotin (Sigma). Cells were washed three times in PBS containing 1% BSA and incubated with phycoerythrin-conjugated Streptavidin (Calbiochem, San Diego, CA). The cells were washed three times as before and resuspended in PBS.

One- or two-colour flow cytometric analyses were performed on a FACStar plus (Becton-Dickinson, Mountain View, CA) flow cytometer.

Immunohistochemical staining of fixed tissues

The tissues were placed for 24–48 hr into a large volume of 4% formaldehyde solution buffered with PBS with occasional changes of fixative to obtain mild but uniform fixation. The fixed samples were routinely processed and embedded into paraffin.

5-μm-thick paraffin sections were mounted on 3-aminopropyltriethoxy silane-coated (Sigma) glass slides and attached by overnight storage at 58°. After deparaffinization endogenous peroxidase activity was blocked by treating the samples with methanol containing 1·5% hydrogen peroxide for 30 min at room temperature. Epitopes were partially unmasked by boiling the sections in 0·1 M sodium citrate buffer three times for 5 min in a microwave oven. Non-specific protein binding sites were blocked with 1% bovine serum albumin in PBS for 30 min. The sections were incubated with the first immunoreagents for 16 hr at room temperature and washed three times in PBS. The staining was developed using the StreptABCopmlex/HRP Duet Kit (DAKO) according to the manufacturer's instructions using 3-amino-9-ethylcarbazole (Sigma) as a red chromogen. In some cases the tissue sections were lightly counterstained with Mayer's haematoxylin. Finally the samples were mounted with glycerine jelly.

RESULTS

The p.aCD43cp serum recognizes human CD43

To obtain an immunoreagent that recognizes the cytoplasmic epitopes of the human CD43, rabbits were immunized with the recombinant cytoplasmic tail of the human CD43. The serum was affinity purified and the antibody fraction that bound to the recombinant protein (p.aCD43cp) was used in subsequent studies.

Lysates of CD43-P3 H1 and $CD43^+$ Jurkat cell lines were used to assess whether the p.aCD43cp serum can be used to detect human CD43 in Western blot experiments. The p.aCD43cp serum was shown to bind a protein with an apparent molecular weight of about 130 kDa in Jurkat cell lines but not in lysates derived from P3H1 cells. Similar results

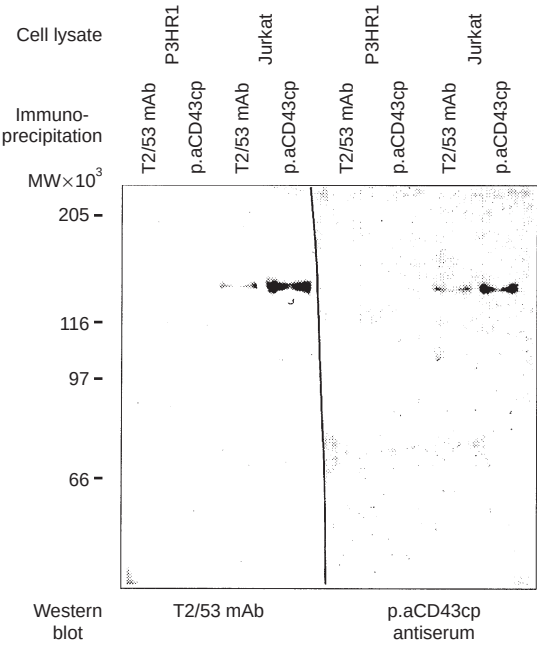

Figure 1. The p.aCD43 cp antiserum recognizes the human CD43 antigen. P3H1 and Jurkat cell lysates were used as CD43 sources for immunoprecipitation with T2/53 or p.aCD43cp. The immunoprecipitated proteins were detected by immunoblotting with T2/53 mAb or p.aCD43cp.

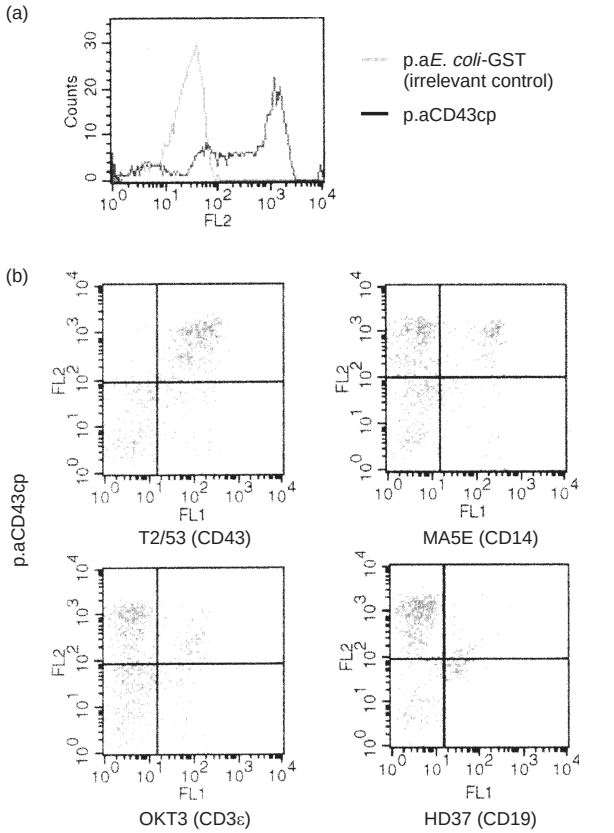

Figure 2. A. The p.aCD43cp antiserum recognizes an antigen in permeabilized human leucocytes as judged by flow cytometry. B. The permeabilized human white blood cells stained by the p.aCD43cp are labelled also by mAb T2/53 (upper left), MA5E (upper right), OKT3 (lower left) but only very weakly by mAb HD37 (lower right) in flow cytometric experiments.

were obtained with mAb T2/53 which is known to recognize an extracellular epitope of the human CD43 glycoprotein (data not shown). The same 130 000-MW protein could be detected by T2/53 mAb in immunoblotting experiments in the p.aCD43cp immunoprecipitates made from Jurkat cell lysates and, similarly, in T2/53 immunoprecipitates by p.aCD43cp (Fig. 1.) providing evidence that the antigens recognized by the two reagents are the same. In contrast, T2/53 and p.aCD43cp immunoprecipitates from P3H1 lysates contained no T2/53 or p.aCD43cp-reactive antigen as judged by immunoblotting.

The affinity-purified p.aCD43cp serum was shown to label permeabilized human leucocytes with varying intensity (Fig. 2a). Expression of epitopes recognized by p.aCD43cp and T2/53 mAb correlated well in the vast majority (>95%) of doubly labelled permeabilized human leucocytes. To determine types of white blood cells expressing epitopes recognized by the CD43cp, a double-coloured flow cytometric system was developed that allowed the simultaneous detection of intracellular and extracellular epitopes in permeabilized cells. Cells that were labelled by p.aCD43cp also expressed epitopes recognized by monoclonal antibodies MA5E (CD14), OKT3 (CD3ε) but not the epitope recognized by the HD37 (CD19) mAb (Fig. 2b).

The p.aCD43cp was shown to be applicable in formaldehyde-fixed tissues after unmasking of the epitopes as described in the Materials and methods. In various fixed human tissues stained by the p.aCD43cp the labelling was confined to haematopoetic cells. In serial lymph node (Fig. 3.) and spleen (data not shown) sections, lymphoid and myeloid cells were found to be stained by p.aCD43cp and T2/53 mAb. These cells were mostly T lymphocytes as revealed by the localization of stained cells to the T-cell zones of the lymphoid tissues and by the comparison of the staining pattern to that obtained with the T-cell specific p.aCD3ε(177–190) serum. Although the staining patterns obtained with p.aCD43cp and mAb T2/53 were similar, p.aCD43cp serum labelled more follicular lymphoid cells than mAb T2/53 or p.aCD3ε(177–190).

The p.aCD43cp serum cross-reacts with the CD43 orthologue of many mammalian species

The p.aCD43cp serum was shown to detect an intracellular antigen or antigens in permeabilized swine and bovine white blood cells (Fig. 4b). To extend the phylogenetic range in which leucocytes express the putative CD43 orthologue that share an epitope or epitopes in common with the cytoplasmic tail of the human CD43, formaldehyde-fixed lymphoid (mammals: thymus, spleen, lymph node, Peyer's patches; birds: thymus, spleen, bursa of Fabricius, Peyer's patches; amphibians and fish: spleen) and non-lymphoid tissues [liver, kidney, heart, lungs (gills), intestine, skin and brain] from fish, amphibians, birds and mammals were stained with the p.aCD43cp serum. Membranes of lymphoid and myeloid cells were stained in most mammal species investigated including all rodents (mouse, rat), even-toed (swine, sheep, goat and cattle) and

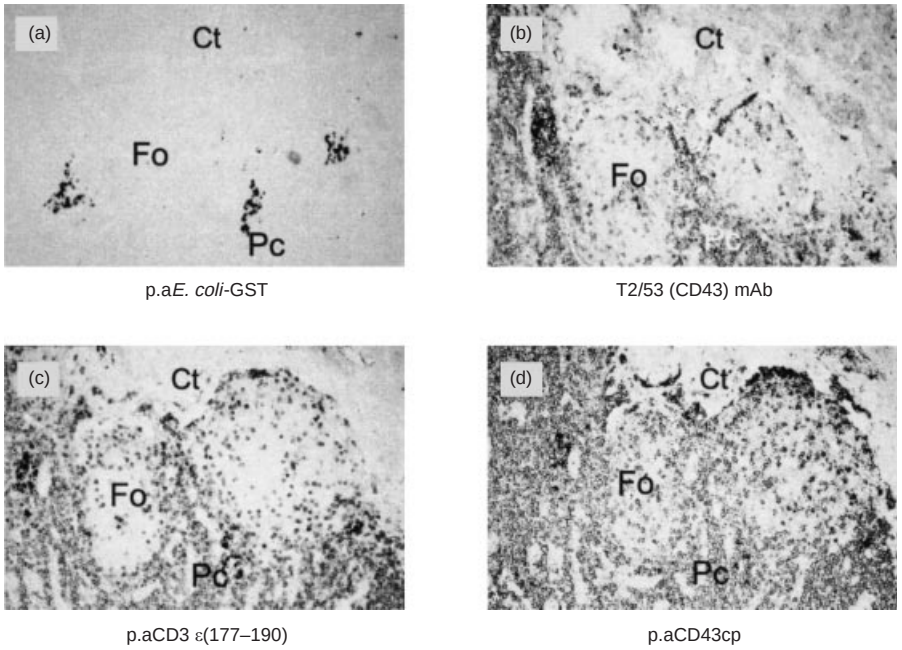

Figure 3. Immunohistochemical staining of serial formalin-fixed human lymph node sections by an irrelevant antiserum (upper left), T2/53 mAb (upper right), p.aCD3ε(177–190) (lower left) and p.aCD43cp (lower right). The main lymph node regions like the capsule consisting of connective tissue, the lymphoid follicles and the paracortex are labelled as Ct, Fo and Pc, respectively.

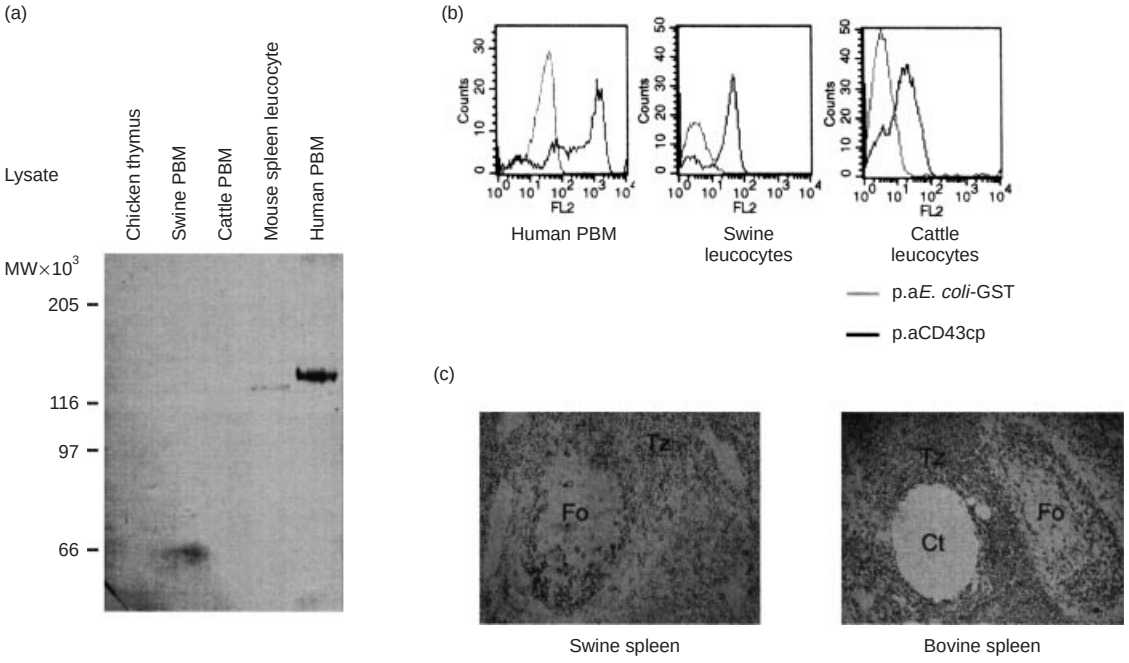

Figure 4. (a) Lysates prepared from chicken thymus, swine PBM, bovine PBM, mouse spleen leucocytes and human PBM, were separated by SDS–PAGE and immunoblotted by the p.aCD43cp antiserum. (b) The p.aCD43cp antiserum labels permeabilized leucocytes from human, cattle and swine. The intensity of the staining is weak in swine and cattle white blood cells as compared with that of human leucocytes. (c) Immunohistochemical staining of formalin-fixed spleen sections from swine (left) and cattle (right) by the p.aCD43cp antiserum. Lymphoid follicles, T-cell zones and connective tissue are marked as Fo, Tz and Ct, respectively.

odd-toed ungulates (horse). Lymphocytes in the T-cell zones of lymphoid tissues were preferentially marked. Staining of serial sections with the p.aCD3ε(177–190) antiserum that is known to recognize CD3ε in many bird and mammal species revealed that the majority of stained lymphocytes were indeed T cells (Fig. 4c). However, a small proportion of CD3ε lymphocytes in follicles of lymph nodes and spleens seemed to express the putative CD43 orthologue. No reactivity in non-

lymphoid tissues was observed. In some mammal species, like carnivores (dog and cat) and in non-mammalian vertebrate taxa, p.aCD43cp failed to detect a CD43 orthologue in the conditions tested.

In contrast to the results obtained in immunohistochemical studies, p.aCD43cp detected a protein that was thought to represent the potential orthologue of the human CD43 only in the leucocyte lysates of rodents but not in other taxa (Fig. 4a) in immunoblot experiments.

Application of the p.aCD43cp serum in veterinary diagnosis

To assess the possible veterinary applicability of the serum, fixed pathological tissue sections were immunostained with the p.aCD43cp immunoreagent. The pathological cases represented a narrow selection of different pathological conditions from various animal species that were previously characterized by conventional histological techniques and by immunostaining with p.aCD3ε(177–190) serum that was previously shown to be applicable for routine veterinary diagnostic purposes.[33] The results are summarized in Table 1. The putative CD43 orthologue was readily recognized by the p.aCD43cp in fixed tissue sections. Infectious diseases were characterized by inflammatory responses that involved the infiltration of the site by $CD3\varepsilon^+ CD43^+$ T lymphocytes or $CD3\varepsilon^- CD43^+$ monocytes and neutrophil granulocytes depending on the type of immune response evoked by the pathogen (Fig. 5a–b). Infiltrating neoplastic cells from bovine lymphoid leukosis cases showed a $CD3\varepsilon^- CD43^+$ immunophenotype with a lymphoid cell morphology (Fig. 5c–d). An uncharacterized lymphoma from swine consisted of mostly $CD3\varepsilon^- CD43^+$ lymphoid cells with a minority of $CD3\varepsilon^+ CD43^+$ T lymphocytes.

DISCUSSION

In this study an affinity-purified antiserum that was raised against the recombinant cytoplasmic tail of the human CD43 protein was shown to identify an antigen with similar biochemical properties and tissue distribution to the CD43.

Permeabilized PBM cells were found to express the epitopes recognized by the p.aCD43cp serum to varying levels; therefore, we determined the cell types that were labelled strongly with p.aCD43cp. T lymphocytes ($CD3\varepsilon^+$ cells) and monocytes ($CD14^+$ cells) were strongly stained, while B lymphocytes ($CD19^+$ cells) expressed p.aCD43cp epitopes in an almost undetectable level. Convincingly, the same cell types were stained in lymphoid tissues in immunohistochemical studies. No reactivity was observed in any cell type of non-haematopoetic origin. As T2/53 mAb that is known to bind a glycosylation-dependent extracellular epitope of the human CD43 behaved similarly to p.aCD43cp in all experiments detailed above, the p.aCD43cp serum is applicable to detect human CD43 protein in native and denatured forms. As p.aCD43cp binds intracellular epitopes that are not glycosylated, this reagent is the first described to recognize CD43 irrespective of its glycosylation status.

Interestingly, some lymphoid cells in the follicles of lymph node and spleen that were labelled by p.aCD43cp were not stained by the T2/53 mAb. Most monoclonal antibodies that bind extracellular epitopes of CD43 recognize epitopes that are dependent on glycosylation and sialation. Glycosylation of this protein is known to be tissue specific and change upon cell activation. It is therefore possible that cells recognized by the p.aCD43cp express a CD43 glycoform that it is not recognized by the T2/53 mAb. Alternatively, p.aCD43cp may cross-react with a membrane protein distinct from CD43 expressed in the T2/53-cells. Either hypothesis awaits confirmation.

Leukosialin as known from human, mouse and rat, contains a phylogenetically conserved cytoplasmic tail; therefore we investigated whether this high level of conservation could be demonstrated with the p.aCD43cp serum at the serological level. In immunoblotting experiments the serum was able to detect a protein corresponding to CD43 in human and mouse but not in other species tested. In immunocytochemical and immunohistochemical experiments, however, the serum recognized T lymphocytes and myeloid cells of many mammalian species.

The results obtained in biochemical and immunocytochemical experiments seem to be somewhat contradictory. As evolutionary distance between species grows the number of shared epitopes is likely to decrease. As protein functions are thought to be coupled to three-dimensional structures, conformational epitopes are more likely to be conserved than linear epitopes. On the other hand, immunoreagents that are applicable for immunoblotting usually recognize linear epitopes that are easily destroyed by single amino acid substitutions. The mean fluorescence intensity of permeabilized white blood cells stained with p.aCD43cp was a lot lower in swine or cattle than in humans. Although it can not be ruled out that this phenomenon is entirely attributable to the lower expression of the CD43 in these species, it is more likely that this is at least partly caused by lower proportions of cross-reactive epitopes. If this is true one might argue that lack of reactivity in species other than humans and rodents in immunoblotting is attributable to the lack of conserved linear epitopes.

The p.aCD43cp serum enabled the identification of the CD43 in many species in which the CD43 protein was previously unknown, providing evidence for the conserved structure of the CD43 cytoplasmic tail. This serum paves the way for the cloning of CD43 in different species which would allow the determination of highly conserved stretches, thereby pinpointing the functionally most relevant regions of this antigen. Current evidence strongly suggests the expression pattern of the CD43 in different mammals is similar. It is not only expressed in the same cell types in all species tested, but it is likely to be regulated similarly during the ontogeny of the immune system. The most characteristic change of CD43 expression is its down-regulation during B-cell development. Many human B-cell lymphomas are $CD43^+$ and are thought to be derived from $CD43^+$ early B cells. Interestingly, some $CD3\varepsilon$ lymphoid tumour cells in bovine lymphoid leukosis and in an uncharacterized lymphoma in swine were stained by the p.aCD43cp reagent, which might indicate that, similarly to human or rodents, early B-cell precursors express CD43 in other mammal species as well. The conserved nature of the cytoplasmic tail together with the finely regulated expression of CD43 suggests that the CD43 protein has an important and specific role in the mammalian immune system.

When immunostaining fixed tissue samples from different pathological cases with p.aCD43cp, $CD43^+$ cells were readily

Table 1. Application of p.aCD43cp in veterinary diagnosis

Disease	Species	Pathogen	Tissue	Pathological alterations	CD3ε$^+$ cells	CD43$^+$ cells
			Inflammatory diseases			
Aujeszky's disease (pseudorabies)	Swine	Herpesvirus	Brain	Lymphocytic perivasculitis	+(T)	+(T)
Maedi	Sheep	Retrovirus	Lungs	Infiltration by mononuclear cells	++(T)	++(T)
			Mammary gland	Infiltration by mononuclear cells	++(T)	++(T)
Caprine arthritis-encephalitis	Goat	Retrovirus	Joint synovial membrane	Infiltration by mononuclear cells	++(T)	++(T)
			Kidney	Infiltration by mononuclear cells	0	0
Sendai-virus pneumonia	Rat	Paramyxovirus	Lungs	Infiltration by mononuclear cells	++(T)	++(T)
Salmonellosis	Swine	*Salmonella typhi-suis*	Lungs	Infiltration by mononuclear cells	±(T)	±(MT)
			Intestines	Infiltration by mononuclear cells	±(T)	++(M,T)
Actinobacillosis	Cattle	*Actinobacillus lignieresii*	Tongue	Granulomatous inflammation	+(T)	++(G,T)
Paratuberculosis	Sheep	*Mycobacterium paratuberculosis*	Intestines	Infiltration by mononuclear cells	++(T)	++(M,T)
	Cattle		Intestines	Infiltration by mononuclear cells	++(T)	++(M,T)
Listeriosis	Sheep	*Listeria monocytogenes*	Brain	Leptomeningitidis	++(T)	++(T)
Leptospirosis	Swine	Leptospira sp.	Kidney	Infiltration by mononuclear cells	++(T)	++(T)
Mycoplasmosis	Horse	Mycoplasma sp.	Lungs	Lymphocytic nodules	++(T)	++(T)
Aspergillosis	Cattle	*Aspergillus flavus*	Lungs	Granulomatous inflammation	0	±(G,M)
Pulmonary nematoditis	Goat	*Dictyocaulus filaria*	Lungs	Infiltration by mononuclear cells	±(T)	±(T)
			Neoplastic diseases			
Lymphoid leukosis	Cattle	Retrovirus	Heart	Malignant lymphoid tumour	0	++(B)
			Liver	Malignant lymphoid tumour	0	++(B)
Malignant lymphoma	Swine	Unidentified	Liver	Malignant lymphoid tumour	+(T)	++(B,T)

Pathological alterations were assessed using standard histological techniques. CD3ε$^+$ and CD43$^+$ cells were detected by immunostaining with p.aCD3ε(177–190) and p.aCD43cp, respectively. CD3ε$^+$ cells T, T cells; CD43$^+$ cells T, T cells; B, early B cells; M, monocytes; G, granulocytes.

Extent of pathological leucocyte infiltration: ++, strong; +, moderate; ±, questionable; 0, none.

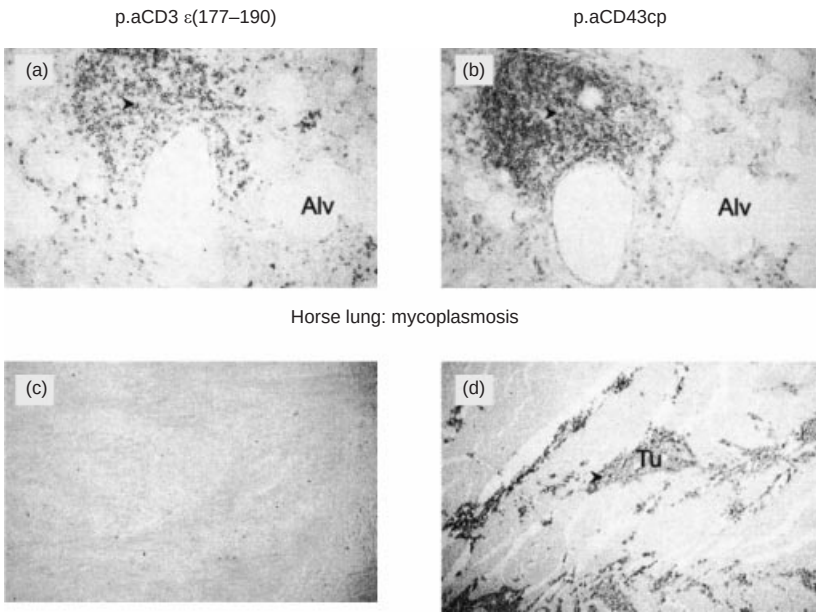

Figure 5. Immunohistochemical staining of fixed tissue sections from various pathological cases by p.aCD3ε(177–190) (a and c) and by p.aCD43cp (b and d). Serial lung sections from a horse that had suffered a pneumonia caused by an uncharacterized mycoplasma strain (a and b) contains a large number of inflammatory leucocytes in the thickened walls of the alveoles (Alv). The inflammatory cells are mainly $CD3\varepsilon^{+}CD43^{+}$ T lymphocytes (a and b) and $CD3\varepsilon^{-}$ $CD43^{+}$ monocytes (b). Serial heart sections from cattle diagnosed with bovine lymphoid leukosis (c and d) contained a large number of tumour cells (Tu). The tumour cells showed a $CD3\varepsilon^{-}$ $CD43^{+}$ immunophenotype (c and d).

labelled, even if the samples had been collected 10 years before the immunohistochemical study. This is relevant as most mAbs recognize epitopes that undergo irreversible denaturation upon fixation,[34] and hence, most mAbs can not be used in routine work and retrospective studies. In our preliminary experiments the p.aCD43cp serum was found to be a reliable reagent for the determination of the haematopoietic origin of different infiltrations and, used together with other reagents, for the serological characterization of normal and pathological lymphoid tissues and lymphoid infiltrations in experimental work and in animal disease.

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