Immunology 1992 **76** 169–173

Tissue localization of conglutinin, a bovine C-type lectin

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Accepted for publication 23 December 1991

SUMMARY

The distribution of bovine conglutinin (BK) in sections of frozen bovine tissues was studied by the indirect immunoperoxidase technique using a monospecific rabbit anti-BK antibody. BK was found in the cytoplasma of all hepatocytes, indicating that the liver is a major site of synthesis of BK. In the germinal centres of the spleen, tonsils and lymph nodes the anti-BK staining was restricted to a population of cells with a distribution and morphology characteristic of follicular dendritic cells. Macrophages in lung and thymus, and glia cells in cerebrum, revealed a granular staining reaction within the cytoplasma. Endothelial cells of blood vessels reacted with anti-BK. The intensity of this reaction varied greatly between the organs, the most pronounced reaction being seen in the glomeruli of the kidney, and in the capillary sinusoides of the cortex of the adrenal gland. The high endothelial venules of lymph nodes and tonsils were also stained. These findings suggest that BK, which until now has been described only as a circulating molecule, may exhibit a biological function within the BK-positive tissues.

INTRODUCTION

Bovine conglutinin (BK) is a calcium-dependent lectin originally described by Bordet and Streng (1909)[1] as a factor which agglutinates erythrocytes preincubated with antibody and complement. Later, it was shown that the high-mannose-group in the alpha-chain of the complement degradation product iC3b is the ligand for BK on complement-coated erythrocytes.[2] The binding can be inhibited by mono- and oligosaccharides especially with molecules containing non-reducing *N*-acetyl-D-glucosamine, L-fucose and mannose.[3,4] Thus BK also has the potential for binding to the cell walls of micro-organisms containing these residues.

Serum BK is a multimeric molecule composed of four structural units each composed of three identical disulphide-linked polypeptides.[5] Judged by gel chromatography serum BK has a M_r of more than 10^6.[6] On SDS–PAGE the single peptide shows an M_r of 43,000 MW under reducing conditions. BK is a hybrid molecule, possessing both a collagenous domain and a carbohydrate recognition domain (CRD) with sequence homology to other C-type lectins.[7]

Abbreviations: BK, bovine conglutinin; CIE, crossed immunoelectrophoresis; CRD, carbohydrate recognition domain; LIE, line immunoelectrophoresis; PBS, phosphate-buffered saline; TBS, Tris-buffered saline.

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BK has a protective effect on mice infected with *S. typhimurium*,[8] and exhibits a complement- and macrophage-dependent antibacterial effect against *Escherichia coli* and *S. typhimurium in vitro*.[9] Structurally BK resembles C1q and binds to the C1q receptor.[10] It has been suggested that the antibacterial effect of BK is mediated via binding to this receptor.

The serum level of BK decreases during acute systemic infections and with calving and abortion,[11] indicating either reduced synthesis, redistribution to cells or tissue, a direct binding to the micro-organisms of BK, or to iC3b deposited on the micro-organisms or immune complexes.

The present study was undertaken in order to acquire information on the tissue distribution of BK by histochemistry on frozen sections from normal cows. This information also provides an indication of possible sites of synthesis of BK.

MATERIALS AND METHODS

Buffers and reagents

Tris-buffered saline (TBS): 140 mM NaCl, 10 mM Tris, 2 mM NaN_3, pH 7·2. Phosphate-buffered saline (PBS): 140 mM NaCl, 10 mM sodium phosphate, pH 7·2. Bovine serum albumin (BSA): Sigma no. B.2518 (Sigma Chemical Co., St Louis, MO). Carbazole: 0·04% 3-amino-9-ethylcarbazole (Sigma no. A-5754) in 0·015% H_2O_2 50 mM sodium acetate buffer, pH 5·0.

Tissues and tissue preparation

Tissues were obtained from a 1-year-old steer immediately after slaughtering. Specimens of 0·25 cm^3 were placed in OCT compound (Miles Scientific, Naperville, IL), snap frozen in dry

ice cooled isopentane and stored at −70°. All tissues were cut in sections of 5 μm.

Antibodies

Antibodies against BK were from rabbits immunized with BK purified as previously described by Friis-Christiansen *et al.*[9] The antiserum was absorbed to monospecificity by the addition of 1/10 vol. of bovine serum in which BK could not be detected by rocket immunoelectrophoresis. The IgG fraction was purified from the monospecific antiserum by ammonium sulphate precipitation followed by ion-exchange chromatography on DEAE-Sephacryl (Pharmacia, Uppsala, Sweden). As a control preparation, the IgG fraction of the same antiserum was used after liquid phase absorption with purified BK. Peroxidase-conjugated swine anti-rabbit IgG (code P217) and rabbit anti-cow serum (code Z184) were from Dakopatts, Copenhagen, Denmark.

Electroimmunoassay

Crossed immunoelectrophoresis (CIE) and line immunoelectrophoresis (LIE) were performed on a 1·5 mm thick 1% (w/v) agarose (Litex HSA, Litex Industry, Copenhagen, Denmark) in 73·2 mM Tris, 24·3 mM sodium barbital, 0·5 mM sodium lactate, 5 mM EDTA, 2 mM sodium azide, pH 8·6. The same buffer was used as electrophoresis buffer. The electrophoresis was run at 2·5 V/cm for 18 hr except for the first dimensional run of CIE which was performed at 10 V/cm, using bromphenol-blue stained albumin as migration marker.

Staining procedure

The indirect immunoperoxidase staining technique was used. Briefly, the procedure was as follows: the dried sections were fixed in acetone for 10 min, washed in TBS, preincubated in 3% w/v BSA in TBS for 10 min, incubated for 1 hr with rabbit anti-BK diluted in 1% BSA in TBS, washed with PBS, incubated for 30 min with peroxidase-labelled swine anti-rabbit IgG diluted 1/50 in 10% v/v human AB serum, washed with PBS and H_2O, incubated with carbazole buffer for 10 min, washed, counter-stained with Mayer's haematoxylin for 5 min, and mounted in Aquamount (BDH Limited, Poole, Dorset, U.K.).

Controls were performed on all tissues by replacing the primary, specific antibody with the same antibody absorbed with purified BK, or by omitting the primary antibody.

RESULTS

Characterization of antibodies

The specificity of the rabbit anti-BK antibody was analysed by CIE. Figure 1 shows the results of CIE analysis of 5 μl bovine serum (first dimension) and 75 μl rabbit anti-BK/cm² agarose (Fig. 1a), or 5 μl rabbit anti-bovine serum/cm² agarose (Fig. 1b) in the second dimension. Only one precipitation arc in the electrophoretic β region was seen using the specific antibody. The antigen in this precipitate was identified as BK by radioiodination of the BK preparation used for electrophoresis, cutting out the precipitate and running it on SDS–PAGE followed by autoradiography. Bands of 300,000 MW (unreduced) and 43,000 MW (reduced) were seen (not shown).

The liquid-phase absorption of the antibody with purified BK was analysed by LIE (Fig. 2). Positive deflection of the line precipitate indicates the presence of antigen in excess, whilst a negative deflection is the result of antibody excess. The ratio between BK and rabbit-anti BK in the IgG used for the preparation of the negative control IgG is indicated in Fig. 2.

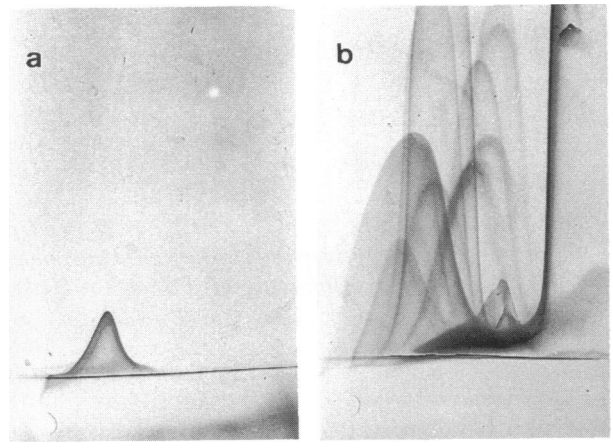

Figure 1. Crossed immunoelectrophoresis using antibody against BK preabsorbed with 1/10 vol bovine serum without detectable BK. Antibody was incorporated in the second dimension gel at high concentration (75 μl/cm² agarose, i.e. diluted 1:2). Five microlitres of bovine serum with high concentration of BK was run in the first dimension (a). (b) CIE of the same bovine serum run against antiserum against total bovine serum proteins.

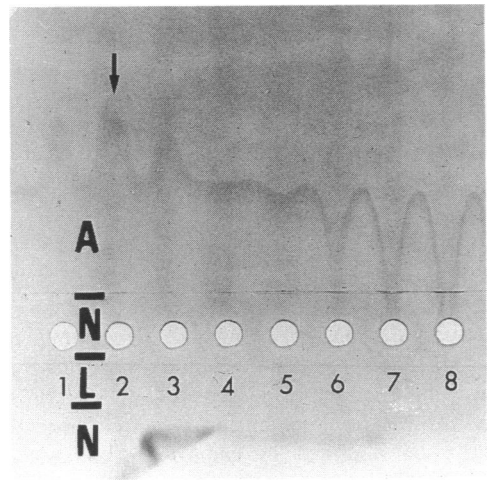

Figure 2. Analysis of the liquid-phase absorption of anti-BK antibody with purified BK using line immunoelectrophoresis. The top gel (A) contained 2 μl antiserum/cm² gel. The line gel (L) contained 0·7 μl bovine serum/cm² gel. N is a neutral gel. The wells contained equal volumes of purified antibody preincubated for 2 hr at 4° with a twofold dilution of purified BK, followed by centrifugation 10^4 ***g*** for 30 min. Negative deflection of the line precipitates indicates an excess of antibody and positive deflection indicates an excess of BK. The ratio in well no. 2 (with BK in excess) was employed in the preparation of the negative control for immunohistochemistry.

Tissue localization of bovine conglutinin

The staining pattern of the bovine liver with anti-BK is shown in Fig. 3a. A granular staining is seen in the cytoplasma of all

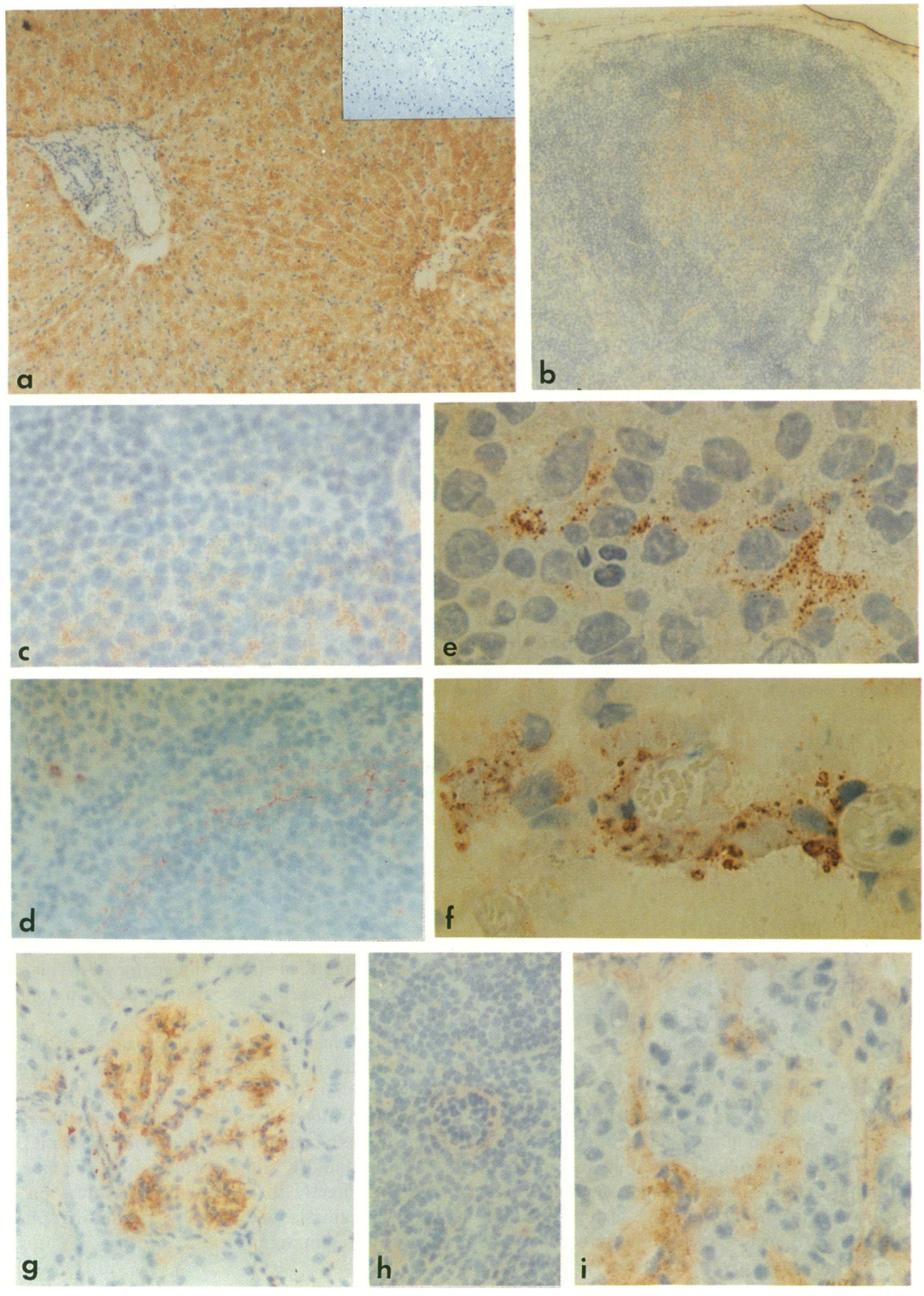

Figure 3. Immunoperoxidase staining for BK with counterstaining with Mayers haematoxylin. (a) Part of a liver lobule. All hepatocytes are uniformly stained in the cytoplasm with the rabbit anti-BK antibody. A positive hepatic portal vein is also shown (× 50). The inset shows liver stained with the same antibody after liquid-phase absorption (× 16). (b) Cortex of a lymph node. The follicular dendritic cells of the germinal centre are positive (× 16). (c) Same as (b) but magnification is greater (× 130). (d) Germinal centre from spleen showing stained cells corresponding to the mandle zone (× 100). (e) Part of a germinal centre from a tonsil showing a single follicular dendritic cell (× 400). (f) Section from bovine lung where a macrophage with distinct reaction corresponding to the phagolysosomes is seen (× 400). (g) Part of the renal cortex, where the glomerulus is positively stained (× 130). (h) The paracortical zone of a lymph node showing a high endothelial venule with positive reaction (× 150). (i) Zona glomerulosa of the adrenal cortex with the capillary sinusoids intensely stained (× 100).

hepatocytes. A moderate staining reaction was also seen of the endothelial cells of the blood vessels, while the connective tissue, biliary ducts and portal tracts showed no staining. The insert in Fig. 3a shows liver stained with the anti-BK antibody after liquid-phase absorption.

The reactions of anti-BK with lymph nodes, spleen and tonsils are shown in Figs. 3b,c,d,e. Figure 3b and c shows a germinal centre from a lymph node with fine granular staining of the follicular dendritic cells. In the germinal centre of the spleen a population of cells with dendritic morphology also revealed intense granular staining. In some centres these positively stained cells were distributed in areas corresponding to the mandel zone (Fig. 3d), whilst in other centres they were localized to the B-cell zone (not shown). Figure 3e shows a single cell in a germinal centre from the tonsils staining positive. The characteristic morphology of a follicular dendritic cell is seen, with euchromatic irregular-shaped nuclei and highly convoluted dendritical processes. The intensity of the staining varied greatly between germinal centres. B and T cells were negative.

In the lung, alveolar macrophages were intensely stained intracellularly in a pattern corresponding to their phagolysosomes (Fig. 3f). Microglia cells in the brain were all moderately stained, whilst all nerve cells were negative (not shown). Weak staining of the endothelial cells of the brain blood vessels was also seen.

The thymic cortex macrophages were lightly stained, and staining was also found corresponding to the Hassal's corpuscles in the thymic medulla (not shown). Here again the endothelial cells were stained.

The glomerulus of the kidney was intensely stained, while the Bowman's capsule and all tubuli were negative (Fig. 3g).

In lymph nodes, tonsils and spleen all endothelial cells including the high endothelial venules were stained but with varying intensity (Fig. 3h). The capillary sinusoids of the adrenal cortex were stained, most intensely corresponding to zona glomerulosa (Fig. 3i).

DISCUSSION

BK is a well-characterized protein and the recently published amino acid sequence indicates that BK is a C-type lectin.[7] C-type lectins are a growing family of proteins characterized by Ca^{2+} dependent binding to carbohydrates and by a carbohydrate recognition domain (CRD) containing 18 highly conserved residues.[12] They are found both as membrane-bound and soluble proteins. Many of the C-type lectins are mosaic proteins containing other functional domains apart from the CRD.[13] Circulating conglutinin has a collagen-like domain, similar to that found in mannan-binding protein, lung surfactant protein A and lung surfactant protein D. Another group of C-type lectins, the selectins, are membrane-bound molecules also possessing an EGF (epidermal growth factor) and several copies of the short consensus repeat motive involved in regulating complement activation.[14] This group of lectins, which include the peripheral lymph node homing receptor, the endothelial cell adhesions molecule and the granule membrane protein-140 are cell adhesion molecules involved in the communication between endothelial cells and leucocytes.

BK has until now only been described as a circulating molecule. In the present study involving immunohistochemical localization of BK we used the IgG preparation of an antiserum against BK that showed monospecificity. When the antibody was analysed in crossed immunoelectrophoresis in high concentration only one precipitation arc was observed in reaction with a bovine serum containing a high concentration of BK. The anti-BK specificity could be removed by absorbtion with purified BK. When this control preparation was used at the same concentration as non-absorbed antibody, no staining of the tissues was seen.

This monospecific anti-BK antibody showed immunoreactivity with follicular dendritic cells (FDC) in the lymphoid follicles of the spleen, tonsils, and lymph nodes. FDC represents a cell type found in the B-cell regions of all lymphoid tissues. They are characterized by the restriction to lymphoid follicles and their ability to trap and retain immune complexes on their surfaces for a long time.[15] Moreover, they have a characteristic morphology possessing highly convoluted cell processes and euchromatic irregularly shaped nuclei.[16] This morphology is identical to the morphology of the BK-positive cells in the germinal centres. Another generally accepted feature of FDC is that they express complement receptors.[17,18] BK on a cell membrane may serve as a complement receptor binding iC3b.

BK immunoreactivity was also found corresponding to endothelial cells. Endothelial cells and FDC have other membrane proteins in common, e.g. the adhesions molecule ICAM-1[14] and major histocompatibility complex (MHC) class II. Another C-type lectin, the selectin ELAM-1, is also localized to endothelial cells, where it promotes adhesion of leucocytes to endothelium inflammation.[19] Macrophages from liver, lung, thymus and spleen also expressed BK immunoreactivity. In some macrophages the staining was localized to the phagolysosomes, indicating that BK had been internalized in the course of phagocytosis. It is not possible to conclude whether circulating BK was bound to a receptor (e.g. the C1q receptor) and then internalized or whether BK by itself is a membrane protein which, after recognition of glycosylated components, was internalized in a manner similar to the macrophage mannose receptor.[20] Other phagocytes, e.g. glia cells, showed a uniform cytoplasmatic staining.

The liver revealed a universal and intense cytoplasmatic staining for BK in almost all hepatocytes. This staining pattern suggests that the hepatocytes may be the major site of synthesis of plasma BK.

In conclusion, the tissue localization of BK in association with follicular dendritic cells, macrophages and endothelial cells indicates that BK may play an important role as a membrane-associated molecule in the immune system. BK may exhibit function as a receptor for complement factor iC3b, but it is also possible that BK plays a role is the first line of defence by binding directly to carbohydrate residues on the surface of micro-organisms and thereby facilitating phagocytosis and antigen presentation.

ACKNOWLEDGMENTS

This work was supported by the Danish Medical Research Council (no. 12-9502), the Danish Agricultural and Veterinary Research Council (13-4464-1), Novo's Fonds Komite and Ingemann O. Bucks Fond. We thank Mrs Anette Kliem and Mrs Lise Schrøder for skilled technical assistance.

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