Immunology 1998 **94** 122–128

Immunohistochemical investigation of the tissue distribution of mannan-binding lectin in non-infected and virus-infected chickens

O. L. NIELSEN,* P. H. JØRGENSEN,* J. HEDEMAND,† J. C. JENSENIUS,‡ C. KOCH§ & S. B. LAURSEN‡ **Danish Veterinary Laboratory, Aarhus, †Danish Institute of Agricultural Sciences, Foulum, ‡Department of Medical Microbiology and Immunology, University of Aarhus, Aarhus, and §The State Serum Institute, Copenhagen, Denmark*

SUMMARY

This paper describes the results of immuno-histochemical staining for chicken mannan-binding lectin (MBL) in formalin-fixed tissue sections from non-infected chickens, and from chickens infected with infectious laryngotracheitis virus (ILTV) or infectious bursal disease virus (IBDV). In the non-infected chickens, MBL was detected in the cytoplasm of a few hepatocytes and in the germinal centres of the caecal tonsils, whereas sections of kidney, heart muscle, spleen, cerebrum, thymus, adrenal gland, bursa of Fabricius, bone marrow and trachea were without staining. In the ILTV-infected chickens, an intense staining reaction for MBL was detected in the cytoplasm of all hepatocytes and on the surface of, and inside, ILTV-infected cells. Also in the IBDV-infected chickens, an intense staining reaction for MBL was detected in the cytoplasm of all hepatocytes. No staining was seen in the follicles of the bursa of Fabricius, but MBL was present in non-identified cells in the interstitium, and in the cytoplasm of macrophage-like cells, located peripheral to the ellipsoid of the spleen. These findings indicate the liver as the primary site of MBL synthesis, and points to up-regulation as a result of the viral infections. The location outside the liver could indicate a role of MBL in the immune defence.

INTRODUCTION

Mannan-binding lectin (MBL) has been isolated from chicken liver[1] and serum,[2] and recently it was purified from chicken serum and characterized.[3] MBL belongs to a group of collagen–lectin hybrid molecules, denoted collectins, which are composed of an N-terminal collagen-like region and a C-terminal C-type carbohydrate recognition domain (CRD). Judged by gel chromatography the intact chicken MBL molecule, like mammalian serum MBL, has a molecular weight of about 750 000. MBL is an oligomer of subunits, each composed of three 30 000–34 000 MW polypeptides joined in a collagen-like triple helix with three globular CRD. The intact, sertiform molecule resembles a bouquet of four to six tulips, analogous to the structure of C1q, the first component of the classical complement pathway. Chicken MBL binds preferentially to *N*-acetyl-D-mannosamine (ManNAc) and a few other monosaccharides in the presence of Ca^{2+}, and is subsequently capable of activating the MB lectin pathway of complement activation.[4]

Mammalian MBL binds to a wide range of micro-organisms, including virus. Clinical investigations suggest that this binding, and the subsequent activation of complement, is of importance for the innate immune defence.[5,6] However, only one experimental *in vivo* study has been reported, and this surprisingly revealed an increase of virus titre in the liver of mice infected with herpes simplex virus (HSV) type 2, when the mice were pre-treated with MBL.[7]

The purpose of the present immunohistochemical study was to examine the tissue location of MBL in non-infected chickens, and to determine the cellular distribution of MBL and possible co-location with virus in infected tissues, taken from experimentally virus-infected chickens suffering from acute disease.

Received 30 June 1997; revised 12 December 1997; accepted 16 December 1997.

Abbreviations: BSA, bovine serum albumin; CRD, carbohydrate recognition domain; ELD_{50}, egg lethal dose at 50% level; HE, haematoxylin and eosin; HIV, human immunodeficiency virus; HRP, horseradish-peroxidase; HSV, herpes simplex virus; IBDV, infectious bursal disease virus; ILTV, infectious laryngotracheitis virus; mAb, monoclonal antibody; ManNAc, *N*-acetyl-D-mannosamine; MBL, mannan-binding lectin; SPF, specified pathogen-free; TBS, Tris-buffered saline.

Correspondence: Dr O. L. Nielsen, Danish Veterinary Laboratory, 2, Hangøvej, DK-8200 Aarhus N, Denmark.

MATERIALS AND METHODS

Chickens and tissue samples

Three normal, non-infected, 8–13-week-old chickens, six infectious laryngotracheitis virus (ILTV)-inoculated, 7-week-old chickens, and six infectious bursal disease virus (IBDV)-inoculated, 4-week-old chickens were examined for the presence of MBL in different tissues. All chickens were of layer

type and were kept under positive pressure in isolators supplied with sterile filtered air.

Tissue sections of kidney, liver, heart muscle, spleen, cerebrum, caecal tonsil, thymus, adrenal gland, bursa of Fabricius, bone marrow and trachea from one non-infected chicken were examined, while the two remaining non-infected chickens were examined for the presence of MBL in the liver (one chicken) and caecal tonsils (one chicken). Also tested were samples of the liver from two chickens and of the trachea from four chickens suffering from acute haemorrhagic tracheitis due to a preceding inoculation (5 and 3 days earlier, respectively) with ILTV, and samples of the liver, bursa of Fabricius and spleen from two chickens, and of the bursa and spleen from one additional chicken, all affected with acute infectious bursal disease (IBD) induced by IBDV inoculation 3 days earlier. Samples of the spleen from three chickens, inoculated 10 days earlier with IBDV, were also examined.

Two serial sections of the spleen from one of the chickens affected with acute IBD were examined for the presence of MBL and vimentin, respectively.

Virus

Each of the ILTV-infected chickens was inoculated intratracheally with 0·4 ml preparation of the A96 strain of ILTV (Central Veterinary Laboratory, Weybridge, UK), containing $10^{3·5}$ egg lethal dose at 50% level (ELD_{50}) and harvested from inoculated specified pathogen-free (SPF) chicken embryos.

The IBDV-infected chickens were inoculated with allantoic fluid harvested at the death of SPF chicken embryos inoculated with the 52/70 strain of IBDV (provided by Dr E. Vielitz, Lohmann Tierzucht, Cuxhaven, Germany). The allantoic fluid contained $10^{3·2}$ ELD_{50}/ml. The chickens received 0·1 ml orally and 0·05 ml in each of the conjunctival sacs.

Chicken MBL

Chicken MBL was partially purified using the first purification step described elsewhere.[3] Briefly, diluted chicken serum was passed through a column containing TSK-75 beads derivatized with ManNAc. Ca^{2+}-dependent ManNAc-binding proteins were eluted with ethylene diaminetetraacetic acid (EDTA).

SDS–PAGE and Western blotting

The partially purified chicken MBL (1 μg) was fractionated under either reducing or non-reducing conditions in sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) on a 4–20% gradient gel (0·75 × 150 × 150 mm), using the Laemmli discontinuous buffer system.[8] Reduced samples were denatured at 100° in 40 mM dithiothreitol, 1·5% (wt/vol.) SDS, 5% (vol./vol.) glycerol, 0·1 M Tris–HCl, pH 8·0, and then alkylated by the addition of 90 mM iodoacetamide (IAA). Non-reduced samples were prepared omitting dithiothreitol. The molecular weight standards were coloured protein molecular weight markers (Rainbow®, cat. no. RPN 755, Amersham, Buckinghamshire, UK). The proteins were transferred to a polyvinylidene difluoride membrane (Immobilon, Millipore, Watford, UK). The filter was blocked for 15 min in Tris-buffered saline (TBS: 10 mM Tris–HCl, 140 mM NaCl, pH 7·4), 0·1% Tween-20, cut in 3-mm strips and incubated with primary antibodies. Monoclonal antibodies (mAb) employed were HYB 182-1, HYB 182-2 and HYB 131-1 (State Serum Institute, Copenhagen, Denmark). HYB 182-1 and HYB 182-2 are directed against chicken MBL, whereas HYB 131-1 (control) is specific for human MBL.[9] All mAb are of the IgG1κ isotype. Also employed was a polyclonal rabbit anti-chicken MBL antibody[3] and normal rabbit IgG, purified from the same rabbit prior to immunization. The antibodies (1 μg/ml TBS, 0·05% Tween-20) were incubated with the Western blotting strips for 2 hr in Octatrays (Pateof, Copenhagen, Denmark). The strips were washed three times in TBS, 0·05% Tween-20 and incubated with either 0·2 μg alkaline phosphatase-conjugated rabbit anti-mouse immunoglobulin (cat. no. D 0314, DAKO, Glostrup, Denmark) or 0·2 μg alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin (cat. no. A 8025, Sigma, St. Louis, MO) per ml TBS, 0·05% Tween-20. After 2 hr incubation, the strips were washed as above and developed with 100 μg nitro blue tetrazolium (cat. no. N 6876, Sigma) and 50 μg potassium-5-bromo-4-chloro-3-indolylphosphate (cat. no. B 6274, Sigma) per ml 2 mM $MgCl_2$, 0·1 M ethanol amine, pH 9·0. Western blotting strips were also stained for total protein using colloidal gold.[10]

Chicken serum MBL

Serum MBL concentration was measured in a sandwich enzyme-linked immunosorbent assay (ELISA). Briefly, microtitre plates (Nunc, Kamstrup, Denmark) were coated with the monoclonal anti-chicken MBL antibody, HYB 182-1. Serum samples were added, and the amount of bound MBL was measured by adding the biotinylated mAb followed by alkaline phosphatase-conjugated avidin (Cat. no. A2527, Sigma, St Louis, MO, USA). The absorption values were measured at 405 nm (para-nitrophenyl-phosphate) and transformed to MBL concentration by means of an internal serum standard, which was calibrated against purified chicken MBL.[3]

Immunohistochemical staining for MBL

A three-layer, indirect immunoperoxidase technique was used. All incubations were performed in a moist chamber at room temperature, unless otherwise specified. Tissue samples were fixed for 3 hr in 10% (vol./vol.) formalin in 60 mM phosphate buffer, pH 7·2, dehydrated, paraffin wax-embedded, cut into 3–5-μm thick sections, mounted on Super Frost Plus slides (Gerhard Menzel, Braunschweig, Germany), dewaxed in paraffin and rehydrated. The tissue sections were then incubated for 5, 10, 20, or 40 min at 37° with 0·1% (wt/vol.) trypsin type II (cat. no. T 8128, Sigma) in 50 mM Tris–HCl, 0·1% $CaCl_2$, pH 7·6. Blocking of endogenous peroxidase activity and non-specific background staining was performed by incubating the sections for 40 min at 37° with 0·1% (vol./vol.) phenyl-hydrazine (cat. no. P 6766, Sigma) in TBS, and for 20 min with 1% (wt/vol) bovine serum albumin (BSA; cat. no. A-7906, Sigma) and 1% (wt/vol.) 'blocking reagent' (cat. no. 1096176, Boehringer Mannheim, Mannheim, Germany) in TBS. Subsequently, the sections were incubated for 1 hr with a mixture of the two monoclonal anti-chicken MBL antibodies, HYB 182-1 and HYB 182-2, each at a concentration of 20 μg/ml TBS, 1% BSA. The sections were then incubated for 1 hr with horseradish-peroxidase (HRP)-conjugated rabbit anti-mouse immunoglobulin (cat. no. P 260, DAKO), diluted 1/100 in TBS, 1% BSA, and for 1 hr with HRP-conjugated swine anti-rabbit immunoglobulin (cat. no. P 217, DAKO), diluted 1/50 in TBS, 1% BSA. Finally, the sections were

stained for 15–45 min with carbazole chromogen (4 mg 3-amino-9-ethylcarbazole, cat. no. A 5754, Sigma, 1 ml *N,N*-dimethylformamide and 150 µl 3% H_2O_2 in 14 ml 0·1 M acetate buffer, pH 5·2, counterstained with Meyers haematoxylin and mounted with Aquamount (BDH, Poole, UK). Between each of the described steps the sections were washed with TBS.

Sections of liver from one of the ILTV-infected chickens were included as positive staining control in each of the test rounds. In addition, two serial sections from all tissues were mounted on each slide. One section was incubated with anti-MBL antibody and the other with an irrelevant monoclonal mouse IgG1κ antibody (cat. no. X0931, DAKO) at the same concentration.

Specificity of the staining with the anti-MBL antibody was analysed substituting the antibody with antibody pre-absorbed with purified MBL. Absorption was accomplished by mixing 4 µg of antibody with 90 µg of pure chicken MBL[3] in 100 µl TBS, 0·05% Tween-20, followed by incubation at room temperature for 1 hr. The purified chicken MBL was prior to absorption denatured at 100° for 15 min.

Immunohistochemical staining for vimentin

Detection of vimentin was performed according to the instructions given by the producer of the formalin grade, anti-vimentin mAb, clone Vim3B4 (cat. no. 11 12457, Boehringer Mannheim). Incubation with an irrelevant monoclonal mouse IgG2a antibody (cat. no. X0943, DAKO), at the same concentration, was used as a negative staining control.

Histopathology

Histopathological examination was performed on haematoxylin and eosin-stained (HE) sections.

RESULTS

Western blotting

Figure 1 shows the characterization by Western blotting of the antibodies employed in this study. The mAb HYB 182-2 and the polyclonal rabbit antibody reacted specifically with both reduced and non-reduced chicken MBL. A double band of 33 000 and 34 000 MW characteristic for reduced chicken MBL[3] was stained with these antibodies. When analysed on non-reduced MBL, a series of high molecular weight bands were stained. HYB 182-1 reacted with non-reduced chicken MBL, only. The controls, using either monoclonal anti-human MBL (HYB 131-1) or preimmune rabbit IgG, were negative. Colloidal gold-stained bands characteristic for chicken MBL as well as bands (28 000 and 65 000 MW, reduced) which could be chicken anti-carbohydrate (ManNAc) antibody.

Specificity

The result of the absorption experiment is shown in Fig. 2(a) and (b). With anti-MBL antibody a granular staining was seen in the cytoplasm of the hepatocytes and in the plasma component of blood located in the lumen of blood vessels, whereas no staining was seen when the same antibody had been absorbed with purified MBL. The liver sections included as positive controls in all the following test rounds always showed granular staining of hepatocytes and staining of plasma. No staining was seen on any of the tissues when the anti-MBL antibody was substituted with an irrelevant antibody of identical isotype and concentration.

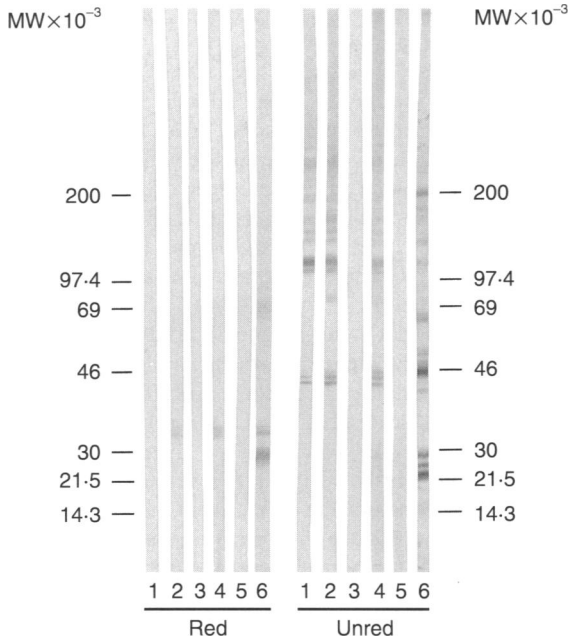

Figure 1. Western blotting analysis of antibodies employed. Partially purified chicken MBL was run either reduced (left) or unreduced (right). Lane 1, mAb anti-chicken MBL (HYB 182-1); lane 2, mAb anti-chicken MBL (HYB 182-2); lane 3, mAb anti-human MBL (HYB 131-1); lane 4, rabbit anti-chicken MBL; lane 5, preimmune rabbit IgG; lane 6, colloidal gold staining of total proteins.

Location of MBL in tissue from non-infected chickens

The liver from two chickens, with MBL serum concentration of 6·8 and 6·3 µg/ml, respectively, were investigated. A granular staining was observed in the cytoplasm of a few hepatocytes in both chickens. However, most of the cells were without staining (Fig. 2c). Staining of lymphoid tissue is shown in Fig. 2(d). MBL was detected in a few cells in the germinal centres of the caecal tonsils from both chickens. Two extremes of cytoplasmic staining were observed. Staining with a reticular distribution (Fig. 2e), or a patchy staining with close apposition to cell nuclei (Fig. 2f). Location of MBL in tissues other than the liver and caecal tonsils was investigated in one chicken, and staining related to cells was not observed in the kidney, heart muscle, spleen (Fig. 4e), cerebrum, thymus, adrenal gland, bursa of Fabricius (Fig. 4c), bone marrow and trachea. However, staining was always present in the plasma component of blood in the lumen of blood vessels.

Location of MBL in tissue from ILTV- and IBDV-infected chickens

The liver from two ILTV- and two IBDV-infected chickens, with MBL serum concentrations of 17·0, 14·3, 18·8 and 40·0 µg/ml, respectively, were investigated. The liver from all infected chickens showed an intense granular staining of the cytoplasm of all the hepatocytes (Fig. 3a). However, staining was more intense in the livers from the two IBDV-infected

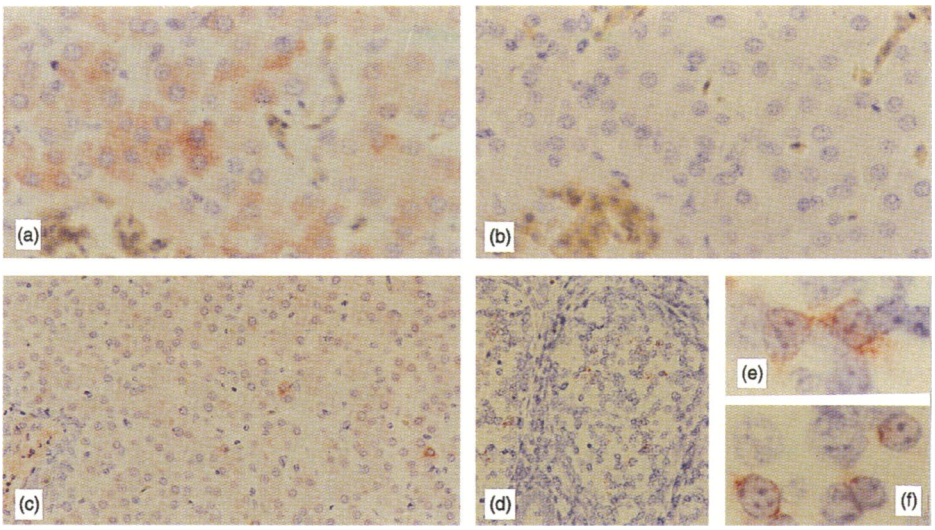

Figure 2. Indirect immunoperoxidase staining for MBL. The sections were stained with mAb anti-MBL, HRP rabbit anti-mouse immunoglobulin and HRP swine anti-rabbit immunoglobulin, counterstained with Meyers haematoxylin. (a) Anti-MBL antibody applied to a liver section from an ILTV-infected chicken gave a granular staining in the cytoplasm of the hepatocytes (×280). (b) Anti-MBL antibody absorbed with purified MBL applied to another liver section from the same chicken. The reaction was abolished (×280). (c) to (f) Tissues from a non-infected chicken. (c) Liver: staining is seen in the cytoplasm of a few hepatocytes (× 140); (d) staining is located to germinal centres of the caecal tonsils (×140); (e) reticular and (f) patchy staining of cells from the germinal centre of the caecal tonsils (×700).

chickens compared to the two ILTV-infected chickens. MBL was detected in ILTV-containing syncytia in the lumen of trachea from four chickens tested. Variable staining reactions were seen. Some syncytia were without staining at all, some showed focal staining related to the surface of the syncytia, while others showed an unevenly distributed staining reaction throughout the syncytium cytoplasm and nuclei (Fig. 3b, c, d and e).

Staining of the bursa of Fabricius from one of the three IBDV-infected chickens examined 3 days after infection and, for comparison, the bursa from the non-infected chicken, are shown in Fig. 4(a), (b) and (c). The bursa from all three IBDV-infected chickens showed staining located almost exclusively to the interstitial tissue, whereas staining in follicles was a rare event. In some instances staining of the interstitium was related to cells. However, the identity of these cells could not be establish. No staining was observed in the bursa from the non-infected chicken.

Staining of the spleen from one of the three IBDV-infected

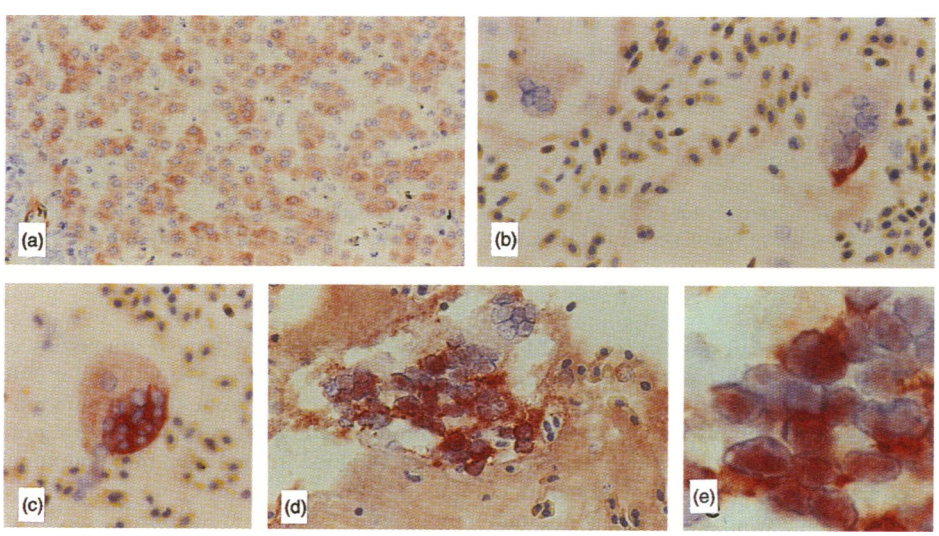

Figure 3. Indirect immunoperoxidase staining for MBL. The sections were stained with mAb anti-MBL, HRP rabbit anti-mouse immunoglobulin and HRP swine anti-rabbit immunoglobulin, counterstained with Meyers haematoxylin. Tissues from an ILTV-infected chicken. (a) Liver: intense granular staining of the cytoplasm of all hepatocytes (×140); (b) tracheal lumen: to the left a syncytium without staining, and to the right a syncytium with focal staining of the surface (×280); (c) tracheal lumen: partial staining of syncytium cytoplasm (×280); (d) tracheal lumen: staining of nuclei from a disrupted syncytium (×280); (e) same as (d) but at higher magnification (×700).

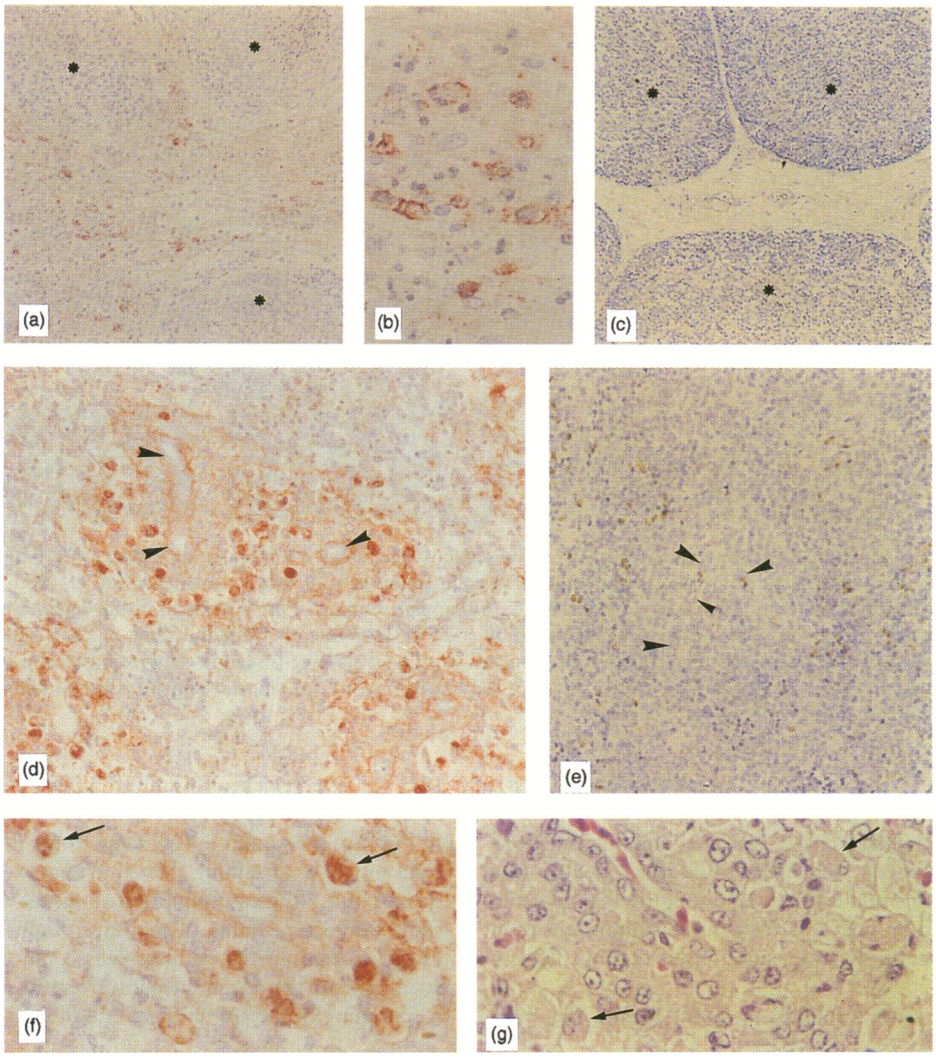

Figure 4. Indirect immunoperoxidase staining for MBL. The sections were stained with mAb anti-MBL, HRP rabbit anti-mouse immunoglobulin and HRP swine anti-rabbit immunoglobulin, counterstained with Meyers haematoxylin. (a), (b), (d) and (f) Tissues from a chicken examined 3 days after IBDV infection; (c) and (e) tissues from a non-infected chicken used for comparison. (g) HE-stained section. (a) Bursa of Fabricius: stained cells are seen in the oedematous interstitial tissue, no staining is seen in the follicles, follicles indicated by * (×70); (b) same as (a) but at higher magnification (×280); (c) bursa of Fabricius: non-infected chicken, no staining seen, follicles indicated by * (×70); (d) spleen: staining of cells located peripheral to the ellipsoid of the sheathed capillaries, endothelial lining indicated by arrowheads (×140); (e) spleen: non-infected chicken, no staining seen, endothelial lining indicated by arrowheads (×140); (f) same as (d) but at higher magnification (×441; stained cells indicated by arrows); (g) HE-stained section of spleen shown in (d) and (f), macrophage-like cells are indicated by arrows (×441).

chickens examined 3 days after infection is presented in Fig. 4(d) and (f). Macrophage-like cells, located peripheral to the ellipsoid of the sheathed capillaries of the white pulp, showed homogeneous to granular, cytoplasmic staining. These cells were seen in the spleen of all three chickens examined, and were identified as degenerated macrophage-like cells in HE-stained tissue sections due to an eccentrically located, pycnotic nucleus and an eosinophilic cytoplasm (Fig. 4g). No staining was seen in the spleen from the non-infected chicken (Fig. 4e), and staining was absent in the spleen from three chickens examined 10 days after IBDV infection.

Two serial sections of the spleen presented in Fig. 4(d) were examined for the presence of MBL and vimentin, respectively. Vimentin was seen in numerous cells throughout the tissue, but not in the cells positive for MBL.

As in sections from the non-infected chicken, all tissues from infected chickens showed staining of the plasma component of blood in the lumen of blood vessels.

DISCUSSION

Chicken MBL has been isolated from liver tissue,[1] and Northern blot analysis of various chicken tissues indicates that MBL is produced only in the liver.[11] In agreement with this we found, in the non-infected chickens, immunoreactivity with anti-MBL antibodies in the cytoplasm of a few liver cells. The

increased number of stained cells and the increased intensity observed in the liver from the ILTV- and IBDV-infected chickens, indicate an up-regulation of synthesis during these acute infections. No preinfection biopsy of the liver from the same chickens was available to verify this alleged up-regulation. Thus, it cannot be excluded that the increased staining observed is a reflection of normal variation in synthesis with no relation to infection. However, the serum concentration of MBL in the four infected chickens was increased two- to sixfold compared to the non-infected birds, and a twofold increase of MBL in serum was induced by experimental IBDV infection in a study comprising 134 chickens, of which half were infected 3 days earlier.[12] This strongly supports the presumption of up-regulation during these acute virus infections.

We also observed reticular staining or patchy staining of cells in germinal centres of the caecal tonsils. The stained cells have not been positively identified, but the reticular staining could be related to the short cytoplasmic cell-processes of follicular dendritic cells.[13]

An initial step in the activation of B and T lymphocytes is the antigen–antigen receptor interaction, and the subsequent cross-linking of receptors. This event results in the spatial concentration of antigen and receptors, which is termed 'capping'.[14] The patchy staining in close apposition to cell nuclei bears some resemblance to the event of 'capping', and could indicate that MBL is involved in the activation of lymphocytes, possibly through the binding of MBL to the antigen.

No staining was seen in sections of kidney, heart muscle, spleen, cerebrum, thymus, adrenal gland, bursa of Fabricius, bone marrow and trachea from the non-infected chicken. If the observed presence of anti-MBL immunoreactivity in germinal centres of the caecal tonsils is a general feature of germinal centres, similar immunoreactivity should be found in the other lymphoid organs, including the spleen. The spleen from the non-infected chicken was without germinal centres. Some germinal centres were present in the spleens from three chickens examined 10 days after the strong antigenic stimulation of IBDV infection. We were, however, unable to detect anti-MBL immunoreactivity in any of these germinal centres. It is possible that the location of MBL to the caecal tonsils is a unique feature, but MBL in lymphoid tissue associated to other epithelia needs further investigation. Interpretation of these results should also take the sensitivity of the assay into account. It is generally accepted that the use of formalin fixation favours morphology over sensitivity whereas the opposite is true for acetone fixation of cryostat sections.

MBL has been demonstrated in several mammalian species, including man[15] and cow.[16] The tissue location of MBL by immunohistochemical techniques has been studied in man only, and the only published investigation reported the presence of MBL in biopsies from livers with different pathological conditions, with no evidence of MBL in normal tissues.[17] However, a recent immunohistochemical study of the tissue location of conglutinin, a bovine collectin, in normal cow showed that the protein was located to the cytoplasm of hepatocytes, the follicular dendritic cells in germinal centres of the spleen, tonsils and lymph nodes, the macrophages in lung and thymus, the glia cells in cerebrum, and the endothelial cells of blood vessels.[18] In contrast to conglutinin, chicken MBL was not seen in macrophages, glia cells and endothelial cells of blood vessels from non-infected chickens.

Infectious laryngotracheitis is caused by a herpes virus that mainly infects the tracheal mucosa, which results in haemorrhagic tracheitis and deaths due to asphyxiation.[19] Viraemia is not seen. Latent infection is established in the trigeminal ganglion after neural migration.[20]

MBL was detected both on the surface, and inside ILTV-infected syncytial cells lying in the tracheal lumen of chickens with virus-induced acute haemorrhagic tracheitis. The implications of these observations remain speculative. MBL could be either taken up from the exudated plasma by the infected cells, or synthesis could be induced in the infected cells by the virus. Human MBL is known to interact with several viruses, i.e. human immunodeficiency virus,[21] influenza,[22] HSV,[7] and it is possible that the observed staining of the ILTV infected cells is due to virus-bound MBL.

Infectious bursal disease is caused by a birnavirus, and has, in contrast to infectious laryngotracheitis, a viraemic phase. The main target cells of IBDV are lymphocytes in follicles of the bursa of Fabricius, in which the virus induces necrosis, but IBDV is also present in infiltrating macrophages and other cells in the follicles and interstitial tissue.[23] Lymphocytes[24] and macrophages[25] of secondary lymphoid organs, including the spleen, also become infected, and IBDV antigen is detected in proliferated macrophages around sheathed capillaries and in the red pulp of the spleen.[26]

MBL was not seen in the follicles, the location of the main target cells for IBDV, but was detected in relation to various non-identifiable cells in the interstitial tissue of the bursa of Fabricius from the IBDV-infected chickens. In addition, MBL was seen in degenerate macrophage-like cells located peripheral to the ellipsoid of the spleen. The detection of MBL in these two organs was related to the IBDV inoculation 3 days earlier, as no staining was seen in either the bursa of Fabricius or in the spleen from the non-infected chicken. However, the possible co-location of IBDV with MBL in the cells needs further investigation.

The location of the MBL-containing cells peripheral to the ellipsoid resembles the location of the ellipsoid-associated cells.[27] Ellipsoid-associated cells are capable of phagocytosis of inert and immunogenic substances;[28] have been implicated in phagocytosis of virus;[29,30] are migratory after activation; and are believed to belong to the group of cells presenting antigen to B and T cells.[28,31,32] Immunohistochemical identification of ellipsoid-associated cells, and other cell-types in the normal spleen, with an anti-vimentin antibody have been described.[33] However, this anti-vimentin antibody showed no reactivity with the cells containing MBL. This indicates that the MBL-containing cells and the ellipsoid-associated cells are two different cell populations, unless the vimentin antigen was destroyed during the process of degeneration. Further characterization of the cells was not attempted.

In conclusion, the detection of MBL in the liver cells of the non-infected chickens, and the increase in staining intensity due to ILTV and IBDV infection, indicates that the liver is the primary site of synthesis, and that these infections induce an up-regulation of synthesis. The location of MBL in germinal centres in the caecal tonsils of non-infected chickens, the ability of MBL to bind to ILTV-infected tracheal cells, and the binding of MBL to macrophage-like cells in the spleen

from IBDV-infected chickens, could indicate that MBL may serve an active role in the immune response.

REFERENCES

1. Oka S., Kawasaki T. & Yaashina I. (1985) Isolation and characterization of mannan-binding proteins from chicken liver. *Arch Biochem Biophys* **241,** 95.
2. Sugii S. & Hirota Y. (1994) Identification and carbohydrate specificity of a chicken serum mannan-binding protein reactive with a Ra chemotype strain of *Salmonella typhimurium*. *J Vet Med Sci* **56,** 747.
3. Laursen S.B., Hedemand J., Thiel S. *et al.* (1995) Collectin in a non-mammalian species: isolation and characterization of mannan-binding protein (MBP) from chicken serum. *Glycobiology* **5,** 553.
4. Thiel S., Vorup-Jensen T., Stover C.M. *et al.* (1997) A second serine protease associated with mannan-binding lectin that activates complement. *Nature* **386,** 506.
5. Holmskov U., Malhotra R., Sim R.B & Jensenius J.C. (1994) Collectins: collagenous C-type lectins of the innate immune defence system. *Immunol Today* **15,** 67.
6. Turner M.W. (1996) Mannose-binding lectin: the pluripotent molecule of the innate immune system. *Immunol Today* **17,** 532.
7. Fischer P.B., Ellermann-Eriksen S., Thiel S., Jensenius J.C. & Mogensen S.C. (1994) Mannan-binding protein and bovine conglutinin mediate enhancement of herpes simplex virus type 2 infection in mice. *Scand J Immunol* **39,** 439.
8. Laemmli U.K (1970) Cleavage of structural proteins during the assembly of the head of Bacteriophage T4. *Nature* **227,** 680.
9. Thiel S., Holmskov U., Hviid L., Laursen S.B. & Jensenius J.C. (1992) The concentration of the C-type lectin, mannan-binding protein, in human plasma increases during an acute phase response. *Clin Exp Immunol* **90,** 31.
10. Schapira A.H. & Keir G. (1988) Two-dimensional protein mapping by gold stain and immunoblotting. *Anal Biochem* **169,** 167.
11. Laursen S.B., Dalgaard T.S., Thiel S. *et al.* (1998) Cloning and sequencing of a cDNA encoding chicken mannan-binding lectin (MBL) and comparison with mammalian analogues. *Immunology* (in press).
12. Nielsen O.L., Sørensen P., Hedemand J.E., Laursen S.B. & Jørgensen P.H. (1998) Inflammatory response of different chicken lines and B haplotypes to infection with infectious bursal disease virus. *Avian Pathology* (in press).
13. Jeurissen S.H.M. & Janse E.M. (1996) The microenvironment of the chicken immune system. In: *Poultry Immunology, Poultry Science Symposium Series* vol. 24, (eds T. F. Davison, T. R. Morris & L. N. Payne), p. 47. CarFax Publishing Company, Oxfordshire.
14. Feldmann M. & Male D. (1989) Cell cooperation in the immune response. In: *Immunology*, edn 2, (eds I. Roitt, J. Brostoff & D. Male), p. 8.1. Gower Medical Publishing, London.
15. Kawasaki N., Kawasaki T. & Yamashina (1983) Isolation and characterization of a mannan-binding protein from human serum. *J Biochem Tokyo* **94,** 937.
16. Holmskov U., Holt P., Reid K.B.M., Willis A.C., Teisner B. & Jensenius J.C. (1993) Purification and characterization of bovine mannan-binding protein. *Glycobiology* **3,** 147.
17. Ryley N.G., Heryet A.R., Lu J., Reid K.B. & Fleming K.A. (1991) Mannan-binding protein in human liver. *J Immunol Methods* **141,** 73.
18. Holmskov U., Teisner B., Pedersen N.T., Laursen S.B., Rasmussen H.B. & Jensenius J.C. (1992) Tissue localization of conglutinin, a bovine C-type lectin. *Immunology* **76,** 169.
19. Jordan F.T.W. (1990) Infectious laryngotracheitis (ILT). In: *Poultry diseases* (ed. F. T. W. Jordan), p. 154. Baillière Tindall, London.
20. Bagust T.J. & Johnson M.A. (1995) Avian infectious laryngotracheitis: virus–host interactions in relation to prospects for eradication. *Avian Pathol* **24,** 373.
21. Ezekowitz R.A., Kuhlman M., Groopman J.E. & Byrn R.A. (1989) A human serum mannose-binding protein inhibits *in vitro* infection by the human immunodeficiency virus. *J Exp Med* **169,** 185.
22. Hartshorn K.L., Sastry K., White M.R. *et al.* (1993) Human mannose-binding protein functions as an opsonin for influenza A viruses. *J Clin Invest* **91,** 1414.
23. Käufer I. & Weiss E. (1976) Electron-microscope studies on the pathogenesis of infectious bursal disease after intrabursal application of the causal virus. *Avian Dis* **20,** 483.
24. Müller H. (1986) Replication of infectious bursal disease virus in lymphoid cells. *Arch Virol* **87,** 191.
25. Tanimura N., Tsukamoto K., Nakamura K., Narita M. & Maeda M. (1995) Association between pathogenicity of infectious bursal disease virus and viral antigen distribution detected by immunohistochemistry. *Avian Dis* **39,** 9.
26. Nunoya T., Otaki Y., Tajima M., Hiraga M. & Saito T. (1992) Occurrence of acute infectious bursal disease with high mortality in Japan and pathogenicity of field isolates in specific-pathogen-free chickens. *Avian Dis* **36,** 597.
27. Olah I. & Glick B. (1982) Splenic white pulp and associated vascular channels in chicken spleen. *Am J Anat* **165,** 445.
28. Olah I., Glick B. & Taylor JR.R.L. (1984) Effect of soluble antigen on the ellipsoid-associated cells of the chicken's spleen. *J Leukocyte Biol* **35,** 501.
29. Jeurissen S.H.M., Scholten R., Hilgers L.A.T., Pol J.M.A. & de Boer G.F. (1989) *In situ* detection by monoclonal antibody D-35.1 of cells infected with Marek's disease virus that interact with splenic ellipsoid-associated reticulum cells. *Avian Dis* **33,** 657.
30. Olah I., Mandi Y., Beladi I. & Glick B. (1990) Effect of human adenovirus on the ellipsoid-associated cells of the chicken's spleen. *Poultry Sci* **69,** 929.
31. Jeurissen S.H.M., Claassen E. & Janse E.M. (1992) Histological and functional differentiation of non-lymphoid cells in the chicken spleen. *Immunology* **77,** 75.
32. Gallego M., del Cacho E. & Bascuas J.A. (1995) Antigen-binding cells in the caecal tonsil and Peyer's patches of the chicken after bovine serum albumin administration. *Poultry Sci* **74,** 472.
33. Olah I. & Glick B. (1994) Anti-vimentin monoclonal antibodies differentiate two resident cell populations in chicken spleen. *Dev Comp Immunol* **18,** 67.