Immunology 1998 **95** 494–500

The working mechanism of an immune complex vaccine that protects chickens against infectious bursal disease

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SUMMARY

The role of immune complexes (Icx) in B-cell memory formation and affinity maturation allow for their potential use as vaccines. Recently, a new immune complex vaccine has been developed that is currently under field trials conducted in commercial poultry. This immune complex vaccine is developed by mixing live intermediate plus infectious bursal disease virus (IBDV) with hyperimmune IBDV chicken serum (IBDV–Icx vaccine). Here we have investigated the infectivity of this vaccine as well as the native IBDV (uncomplexed) vaccine in terms of differences in target organs, in target cells and speed of virus replication. At various days after inoculation on day 18 of incubation (*in ovo*) with either one dose of virus alone or the IBDV–Icx vaccine, the replication of IBDV and the frequency of B cells and other leucocyte populations were examined in the bursa of Fabricius, spleen, and thymus using immunocytochemistry. With both vaccines, IBDV was detected associated with B cells, macrophages and follicular dendritic cells (FDC) in bursa and spleen, although complexing IBDV with specific antibodies caused a delay in virus detection of about 5 days. Most remarkable was the low level of depletion of bursal and splenic B cells in IBDV–Icx vaccinated chickens. Furthermore, *in ovo* inoculation with the IBDV–Icx vaccine induced more germinal centres in the spleen and larger amounts of IBDV were localized on both splenic and bursal FDC. From these results we hypothesize that the working mechanism of the IBDV–Icx vaccine is related to its specific cellular interaction with FDC in spleen and bursa.

INTRODUCTION

In various species, including chickens, immune complexes (Icx) are formed during the immune response to an introduced antigen by binding of newly formed specific antibodies to that antigen.[1] A small proportion of the Icx is trapped in B-cell follicles on the cell processes of follicular dendritic cells (FDC) and can be retained there for a long period of time[2] via binding to Fc receptors[3] and complement C3 receptors.[4] Antigen preserved in this way is believed to play a crucial role in the generation of memory B cells[1,5] and the maintenance of long-term humoral immune responses.[2] In addition, immune complexes bound to FDC are involved in the selection of B lymphocytes that have undergone affinity maturation.[6] *In vitro* preformed Icx have been shown to be 100 times more efficient in inducing humoral immune responses *in vivo* than the native protein antigen.[7] This specific potential of Icx has suggested their use as vaccine antigens.

Recently, a vaccine has been developed consisting of Icx formed by mixing a certain amount of specific antibodies obtained from the serum of hyperimmunized chickens with live infectious bursal disease vaccine virus (IBDV).[8] IBDV is a pathogen of major economic importance to the world's poultry industries. The virus is a double stranded RNA virus belonging to the Birnaviridae family. It causes severe immunodepression in young chickens by destroying the developing B lymphocytes in the bursa of Fabricius and thus diminishing the peripheral pool of reactive B cells.[9] IBDV-induced immunodepression increases the incidence of diseases caused by opportunistic pathogens and prevents young chickens from responding optimally to routinely used vaccines.

The major advantage of the newly developed IBDV–Icx vaccine currently used in field trials, is that it is suitable for injection in fertilized eggs at day 18 of incubation with the Inovoject® machine, currently used for vaccination against Marek's disease virus, and that it is insensitive to maternal antibodies.[10] Furthermore, the efficacy of this new vaccine at challenge is identical or better than that induced by classic vaccines against infectious bursal disease.[10]

A detailed understanding of the mechanism of action of

Received 16 April 1998; revised 14 July 1998; accepted 14 July 1998.

Abbreviations: FDC, follicular dendritic cell; GC, germinal centre; IBDV, infectious bursal disease virus; Icx, immune complex; mAb, monoclonal antibody; PELS, peri-ellipsoidal lymphocyte sheath; p.i., postinoculation.

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this type of vaccine is lacking. It may be hypothesized that the IBDV-specific antibodies in the Icx shield the vaccine IBDV from maternal antibodies and other mechanisms that lead to reduction of the viral load available for induction of the immune response. On the other hand, it is also possible that the complex formation with IBDV-specific antibodies causes a specific localization of IBDV in privileged sites of the immune system, i.e. in germinal centres. This latter phenomenon has been demonstrated in chickens, where Icx specifically localize on FDC in germinal centres.[11] In chickens, the bursa of Fabricius comprises cell types that resemble FDC in their staining pattern with specific monoclonal antibodies and in the capacity of trapping of Icx on their surface.[12] Whether these bursal dendritic cells have a function similar to that of germinal centre FDC in selection of the developing B cells in the bursa is presently unknown.

In this study, the working mechanism of the IBDV–Icx vaccine was investigated by comparing the infectivity of the IBDV–Icx and the virus alone at various timepoints after *in ovo* injection. The following points were investigated: (1) the target organs for virus replication, (2) the timepoints of virus replication, and (3) the cell populations interacting with IBDV. By comparing the results obtained with native IBDV and immune complexed IBDV, it was shown that IBDV–Icx cause less B-cell depletion at a later timepoint and a more extensive induction of new germinal centres where IBDV–Icx localize on FDC.

MATERIALS AND METHODS

Animals

Specific pathogen-free eggs (ID-DLO, Lelystad) were injected manually at day 18 of incubation according to a standard protocol. In brief, a small hole was drilled in the blunt end of the egg. With a 23 gauge, 2·5-cm needle, the inoculum was injected under the air-cell membrane using the full length of the needle. The hole was closed with a drip of glue and the eggs were incubated as usual. After hatching, chicks of the different groups were housed in separate boxes. At 3, 5, 7, 10, 14, 17, and 21 days after inoculation, three to four chicks per group were decapitated. Subsequently the bursa of Fabricius, spleen, and thymus were frozen in liquid nitrogen, and stored at $-20°$.

IBDV vaccines

The IBDV–Icx (batch 52410) vaccine and the IBDV vaccine were supplied by Dr P. Lehrbach (Fort Dodge Australia). The virus strain is strain V877 IBDV.[13] The IBDV–Icx vaccine was prepared by mixing the native virus V877 with IBDV-specific hyperimmune serum. The neutralizing activity of the hyperimmune serum was determined using a virus neutralization assay described by Haddad *et al.*[14] Both vaccines were supplied freeze dried and first resuspended in 25 ml 0·9% NaCl solution, and then before use diluted 1:20. Eggs were injected with 100 µl vaccine suspension containing one dose. One dose of both vaccines contained 150 EID_{50} (mean embryo infective dose) virus. Control eggs were injected with 100 µl 0·9% NaCl solution.

Immunohistochemistry

Cryostat sections of 8 µm thickness were collected on slides, assuring that sections of age-matched control, IBDV and IBDV–Icx vaccinated chickens were assembled on the same slide. Slides were air-dried and fixed in pure acetone for 10 min. Then sections were incubated with monoclonal antibodies in an appropriate dilution for 45 min at RT. Slides were rinsed three times in phosphate-buffered saline. For single staining, sections were subsequently incubated with peroxidase-labelled rabbit antimouse immunoglobulin (Dako, Glostrup, Denmark). After 30 min slides were rinsed again. Peroxidase activity was developed with a solution of 0·5 mg 3,3′-diaminobenzidine-tetrahydrochloride (DAB; Sigma Chemicals, St.Louis, MO) and 0·01% H_2O_2 per ml Tris–HCl buffer (0·05 M, pH 7·6).

For double staining, sections were simultaneously incubated with two first-step antibodies with different isotypes and subsequently incubated with goat conjugates specific for mouse immunoglobulin G isotypes IgG1 and IgG2b labelled with either peroxidase or with alkaline phosphatase (Southern Biotechnology Associates, Birmingham, AL). After rinsing, peroxidase activity was developed with 0·01% H_2O_2 in a solution of 0·4 mg 3-amino-9-ethylcarbazole (Sigma) per ml sodium acetate buffer (0·05 M, pH 5). Alkaline phosphatase activity was developed in a solution of 40 ml Tris–HCl buffer (0·1 M, pH 8·5) containing 5 mg naphthol AS-MX phosphate (Sigma) predissolved in 250 µl *N*-dimethylformamide and 10 mg Fast blue BB base predissolved in 500 µl 1 N HCl, 2% $NaNO_2$. Control slides were treated similarly, except that the first incubation step with monoclonal antibodies was omitted.

Monoclonal antibodies

The following monoclonal antibodies (mAb) specific for chicken cell populations were used: HIS-C7, specific for CD45 on leucocytes;[15] HIS-C1, specific for B lymphocytes;[15] HIS-C12, specific for IgM;[15] CVI-ChIgG-47.3, specific for IgG;[15] CD3, specific for T lymphocytes (SBA); CVI-ChNL-68.1, specific for mononuclear phagocytes;[16] CVI-ChNL-74.2, specific for red pulp macrophages and macrophages around the B-cell sheaths;[17] and CVI-ChNL-74.3, specific for follicular dendritic cells and their precursors.[17,18] In addition, mAb 17-82 specific for VP2a/2b of IBDV was used for single and double stainings to detect presence of IBDV virus particles and VP2 protein.[19]

RESULTS

IBDV virus replication

The virus replication after vaccination with IBDV and IBDV–Icx was investigated in the bursa of Fabricius, spleen and thymus at various timepoints (Table 1). mAb 17-82, specific for IBDV, did not show any non-specific background staining in unvaccinated chickens. In vaccinated chickens, this anti-IBDV mAb stained large round structures of about cell size or bigger. It also stained very small grains, that may represent virus aggregates and inclusions of viral proteins.

IBDV replication was detected from day 5 p.i. in IBDV-vaccinated chickens and from day 7 (one chicken) or day 10 p.i. in IBDV–Icx-vaccinated chickens. Although in both vaccine groups at the peak of viral replication almost every bursal follicle contains IBDV, it is remarkable that the staining pattern in the IBDV-vaccinated chickens is more diffuse and smearlike, whereas in IBDV–Icx-vaccinated chickens the staining is restricted to single cells. In the spleen of IBDV–Icx-

Table 1. Detection of IBDV virus in lymphoid organs at various timepoints after inoculation

Days after inoculation	Age of chicks	Vaccine group	Bursa	Spleen	Thymus
3	1	IBDV	–	–	–
		IBDV–Icx	–	–	–
		control	–	–	–
5	3	IBDV	+++	+++	++
		IBDV–Icx	–	–	–
7	5	IBDV	+++	+++	++
		IBDV–Icx	±	±	–
		control	–	–	–
10	8	IBDV	+++	+++	+++
		IBDV–Icx	+++	+	++
		control	–	–	–
14	12	IBDV	+	±	++
		IBDV–Icx	++	±	+
17	15	IBDV	±	±	+
		IBDV–Icx	++	±	+
21	19	IBVD	±	±	±
		IBDV–Icx	±	±	±
		control	–	–	–

–, virus not detected; ±, few small spots of virus detected; +, isolated islands of virus detected comprising less than 20% of the organ section; ++, virus detected in about 50% of the organ section; +++, virus detected in the whole organ section

vaccinated chickens, most IBDV was located in germinal centres from day 10. In IBDV-vaccinated chickens, germinal centres were absent, as in the control chickens, and IBDV was detected in the peri-ellipsoidal areas.

IBDV replication was simultaneously detected in bursa, spleen and thymus, although the replication seemed to proceed faster in the bursa. When the numbers of B lymphocytes decreased, the relative amount of detectable IBDV also decreased, although in all three organs IBDV remained detectable for longer periods.

Influence of IBDV replication on various cell populations

B lymphocytes (mAbs HIS-C1, HIS-C12, and CVI-ChIgG-47.3). The influence of vaccination with IBDV and IBDV–Icx vaccines on B lymphocytes in the bursa, spleen and thymus is represented in Table 2. After IBDV vaccination, depletion started around day 5, and after IBDV–Icx vaccination around day 10 after vaccination. These time kinetics coincided with the virus replication. Both IBDV and IBDV–Icx vaccine caused a depletion of B cells in bursal follicles, splenic peri-ellipsoidal lymphocyte sheaths and thymic medulla. The depletion caused by IBDV alone, however, was much more severe than that of IBDV–Icx at any timepoint. In double stained sections, a high correlation was found between B lymphocytes and IBDV (Fig. 1a,b). The numbers of double stained cells were lower than expected, probably because replication by IBDV caused a rapid cell death and thus loss of cellular determinants. In the bursa of Fabricius major differences in the follicles could be seen after IBDV vaccination. First, follicles had a normal appearance with medulla and cortex without any IBDV. Then single follicles contained a limited amount of IBDV in the medulla and still contained normal numbers of B cells. In a later phase, the whole follicle was fully stained for IBDV and hardly any B cells could be detected. During the last phase, no B cells were present, whereas only limited amounts of IBDV were found (Fig. 1a). In contrast, in IBDV–Icx-vaccinated chickens, the last phase of infection never occurred as the bursal follicles always continued to contain some B cells even at day 10 when depletion was most severe (Fig. 1b). In IBDV–Icx-vaccinated chickens a rapid repopulation of the follicles was seen seven days later, which was not seen until day 21 after IBDV vaccination.

The most conspicious difference between IBDV and IBDV–Icx, however, was the number of germinal centres filled with B lymphocytes that developed in the spleen (Table 2). From day 10 after vaccination, their number was much higher in IBDV–Icx-vaccinated chickens (Fig. 1f) than in the IBDV-vaccinated chickens (Fig. 1e), where they were absent as in the control chickens. These newly induced germinal centres were already seen at day 10 and 14 after inoculation, when peri-ellipsoidal B lymphocytes were still depleted.

T lymphocytes (mAb CD3). Small numbers of CD3-positive T lymphocytes were found in bursal follicles in control chickens at all timepoints examined and at 3 days after vaccination with IBDV and IBDV–Icx vaccines. From the moment IBDV replication started, however, T lymphocytes increased rapidly in both bursal follicles and splenic peri-arteriolar areas. No correlation was seen between the localization of T lymphocytes and IBDV. Particularly at day 10 in IBDV-vaccinated chickens, T lymphocytes formed a dense ring at the outer side of bursal follicles. After day 10, the relative frequency of T lymphocytes decreased, although their number remained much higher in vaccinated chickens than in control chickens. In IBDV–Icx-vaccinated chickens dense clusters of T lymphocytes were located in the interfollicular area at day 17 and 21 after vaccination. In contrast to bursa and spleen, no differences in the number of T lymphocytes were observed in the thymus.

Mononuclear phagocytes (mAbs CVI-ChNL-68.1 and CVI-ChNL-74.2). In control chickens, single mononuclear phagocytes were found in the connective tissue outside the bursal follicles, in the outer border of the cortex and in the entire medulla of the bursal follicles. From day 7 in IBDV- and from day 10 in IBDV–Icx-vaccinated chickens, however, distinct changes were seen. Concurrent with the depletion of the follicles, an increase in number of mononuclear phagocytes was detected, and simultaneously the staining intensity of the cells increased. The distribution of macrophages changed such that the cells were localized mainly in the outer part of the follicles. No major differences were seen between both vaccinated groups. In the spleen a similar development was seen consisting of increased staining intensity with simultaneous loss of the normal localization pattern. In both vaccinated groups, double staining was detected of macrophages and IBDV.

Follicular dendritic cells (mAb CVI-ChNL-74.3). FDC formed a loose regular network in bursal medullas of control chickens at all timepoints. At day 10 after vaccination with

Table 2. Changes in numbers of B lymphocytes after vaccination with IBDV and IBDV–Icx

Days after inoculation	Age of chicks	Vaccine group	Bursa	Spleen		Thymus
				PELS	No of GC*	
3	1	IBDV	=	=	0	=
		IBDV–Icx	=	=	0	=
5	3	IBDV	< <	< <	0	< <
		IBDV–Icx	=	=	0	=
7	5	IBDV	< < <	< < <	0	< < <
		IBDV–Icx	=	=	0	=
10	8	IBDV	< < <	< < <	0	< <
		IBDV–Icx	< <	<	9±8	<
14	12	IBDV	< < <	< <	2±3	<
		IBDV–Icx	< <	<	6±3	=
17	15	IBDV	< <	<	1±1	<
		IBDV–Icx	<	=	10±5	=
21	19	IBVD	<	<	1±1	<
		IBDV–Icx	<	=	7±5	=

*, The mean number (± SEM) of germinal centres (GC) per spleen section.
=, Same number and distribution of B lymphocytes as in age-matched control chickens.
<, 25% or less of the B lymphocytes were depleted compared to age-matched control chickens.
< <, About 50% of the B lymphocytes were depleted compared to age-matched controls.
< < <, Almost all B lymphocytes were depleted compared to age-matched controls. PELS, peri-ellipsoidal lymphocyte sheath

IBDV–Icx, FDC were detected as small clusters of cells with a very intense staining, probably due to shrinkage of the follicles by the depletion of B lymphocytes (Fig. 1d). The main difference between IBDV and IBDV–Icx-vaccinated chickens was that bursal FDC were totally absent in IBDV-vaccinated chickens for several days (Fig. 1c), whereas they remained detectable, although at low numbers, in IBDV–Icx-vaccinated chickens (Fig. 1d). Double staining of follicular dendritic cells and IBDV showed that IBDV was present as a very thin layer on the cell surface (Fig. 1f). A similar staining pattern was seen after double staining of chicken IgM or IgG and IBDV, indicating that the IBDV is trapped in immune complexes on the surface of FDC. In IBDV-vaccinated chickens, this staining was sporadically seen in spleen and bursa at days 17 and 21 after vaccination (Fig. 1e). In contrast, double staining for folliclar dendritic cells and IBDV was abundantly present in IBDV–Icx-vaccinated chickens in both bursa and spleen from day 10 (Fig. 1d,f).

From the moment the follicles became repopulated again, the FDC staining pattern became comparable to that of control chickens, although the size of the follicles and thus the relative frequency of FDC remained much smaller. In the spleen, hardly any staining of FDC and their precursors was seen in control chickens. From day 10 after vaccination with IBDV–Icx, clusters of FDC were detected in germinal centres near splenic arteries, whereas such clusters did not develop in IBDV-vaccinated chickens. Even at day 21, hardly any distinct germinal centres with FDC were seen in the spleens of IBDV-vaccinated chickens (Fig. 1e), whereas they were clearly present in spleens of IBDV–Icx-vaccinated chickens (Fig. 1f).

DISCUSSION

In this study, the effects of *in ovo* inoculation using either IBDV alone or complexed with specific neutralizing antibodies (IBDV–Icx), have been investigated with respect to virus replication and lymphoid and non-lymphoid cell populations. Three aspects of the virus infectivity were clearly different between the IBDV and the IBDV–Icx vaccine: the onset of viral replication, the severity of B lymphocyte depletion, and the induction of germinal centres with immune-complex trapping FDC. Immune-complex formation of IBDV with specific antibodies caused a delay of 5 days in onset of virus replication, although both vaccines were injected at day 18 of incubation. As both vaccines had the same amount of the same virus, the delay in viral replication can not be caused by a different initial amount of virus. The question as to the mechanism by which IBDV-specific neutralizing antibodies caused this delay in virus replication is difficult to answer, because it is unknown whether the antibody dissociates from the virus or is degraded *in vivo*, or whether escape mutants arise from the virus. To investigate whether the initial localization of IBDV–Icx is different from native IBDV at early timepoints, e.g on FDC in bursal follicles and germinal centres, it will be necessary to visualize both vaccine viruses during the time between injection and first replication. In this study, we could visualize IBDV only after viral replication had occurred. The failure of early detection in the present study is probably a result of the low amounts of IBDV antigen injected in addition to the relative insensitivity of the immunoenzyme detection method[20] as compared to autoradiographic methods[5] or *in situ* polymerase chain reaction (PCR).

After vaccination with IBDV–Icx, B-cell depletion from the bursa of Fabricius was remarkably less severe than after vaccination with IBDV alone, because at all timepoints in the IBDV–Icx vaccinates, small clusters of B lymphocytes could be detected in some bursal follicles. These results are remarkable because a delay of 5 days in the onset of viral replication

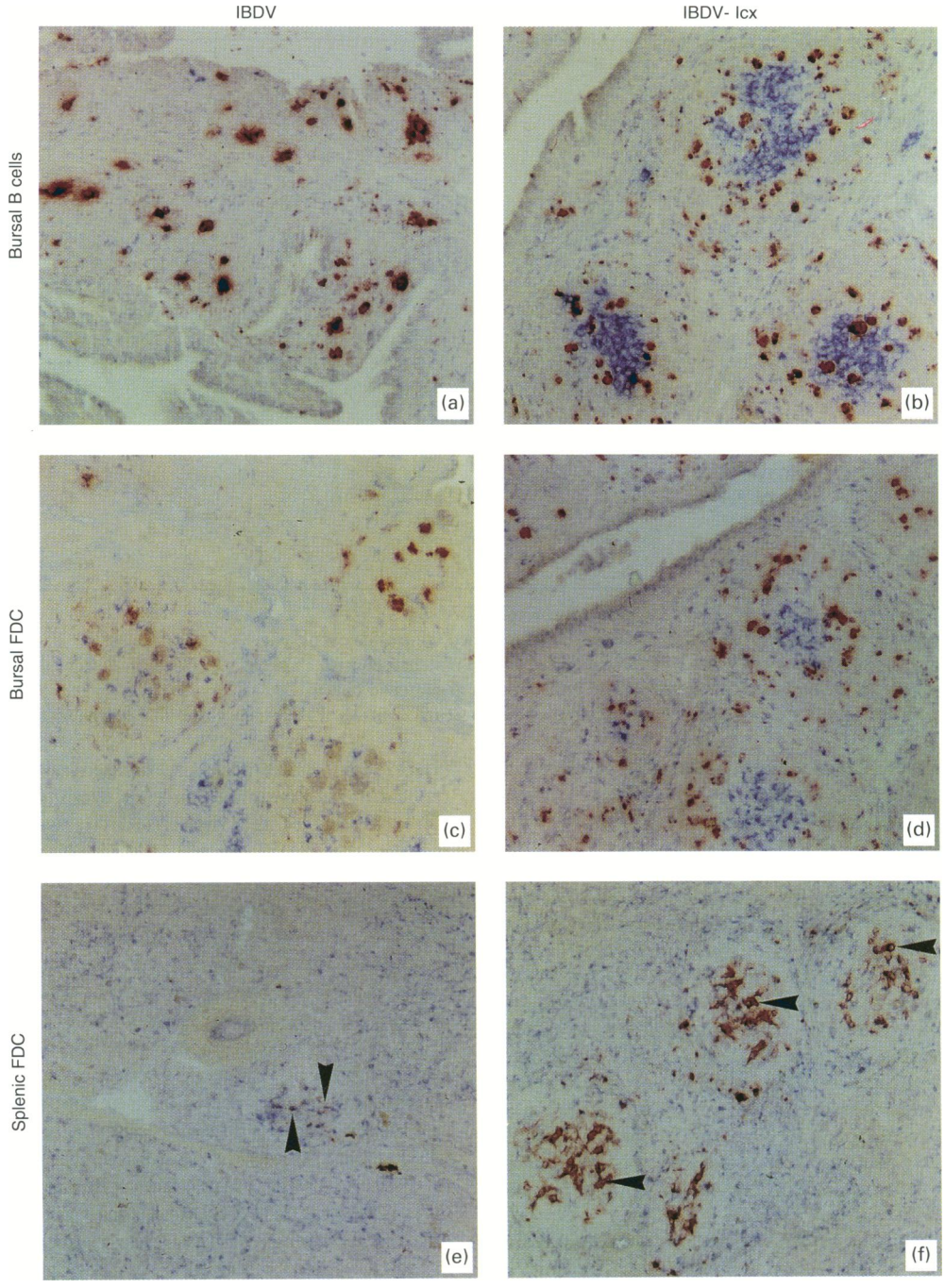

Figure 1. Bursa of Fabricius (a–d) and spleen (e, f) of chickens vaccinated with native IBDV (a, c, e) or IBDV-Icx (b, d, f) vaccine. Magn. ×200. (a) At day 10 after inoculation with IBDV, IBDV (red) was detected in the follicles that were depleted of IgM-positive B lymphocytes (blue). (b) At day 10 after inoculation with IBDV–Icx, most follicles still contained IgM-positive B lymphocytes (blue) intermingled with IBDV aggregates (red). (c) At day 10 after inoculation with IBDV, hardly any follicular dendritic cells (blue) or follicle structures were left, whereas IBDV (red) was still detected. (d) At day 14 after inoculation with IBDV–Icx, still intact follicular dendritic cells (blue) were found next to IBDV replicating cells (red). (e) At day 21 after inoculation with IBDV, only a sporadical germinal centre was detected with IBDV (red) trapped in immune complexes on follicular dendritic cells (blue; arrows). (f) At day 17 after inoculation with IBDV Icx, many germinal centres were detected with immune complexes trapped on FDC (arrows) after double staining with anti-FDC (blue) and anti-IBDV (red).

after vaccination with IBDV–Icx also causes the number of B cells in the bursa available for infection to be much higher. It is well established that older birds (at 3–8 weeks of age) are more susceptible to disease than young chickens (less than 2 weeks of age), because only the older birds show clinical signs of disease.[21] The increased susceptibility has been suggested to be due to the higher number of target cells in the bursa available for viral replication.[22] Another explanation of

the age-related susceptibility is the disability (prior 2 weeks of age) or ability (after 3 weeks of age) of chickens to produce specific antibodies that subsequently form immune complexes and immune-complex disease.[23] This explanation is, however, contradicted by our results where preformed immune complexes decreased the signs of disease. Therefore, it seems that complexation of IBDV with antibodies offers protection to at least some B lymphocytes against the lytic effect of the virus. Protection against IBDV lysis at the cellular level can theoretically be explained by the fact that some of the antibodies bind to the viral sites that would normally interact with the virus receptors on the surface of B cells that are in the direct neighbourhood of FDC with trapped IBDV–Icx.

The most striking difference between the IDBV and the IBDV–Icx vaccine was seen after staining for bursal FDC. In chickens infected with IBDV alone almost all bursal FDC have disappeared after 10 days p.i., whereas in IBDV–Icx-infected animals bursal FDC were always detected. In these chickens, IBDV complexed in immune complexes were located on bursal FDC at later timepoints. The changes seen in a cell population such as FDC could be caused by several mechanisms. It could be directly caused by interaction between this cell type and the virus, but it could also indirectly be caused by the depletion of B lymphocytes and the associated changes in the surrounding microenvironment. With respect to follicular dendritic cells, there is ample evidence that these cells need an intact microenvironment of B lymphocytes to display a normal appearance and function during ontogeny and adulthood.[24]

Major differences between IBDV and IBDV–Icx vaccination were also seen in the spleen. From day 10 p.i. with IBDV–Icx, new germinal centres were induced which contained FDC with IBDV immune complexes trapped on their surface. In contrast, IBDV-inoculated animals hardly contained germinal centres in the spleen even at later timepoints. In contrast to mammals where germinal centres develop in primary B-cell follicles, chicken germinal centres develop in the T-cell areas next to bifurcations of arteries around FDC precursors.[17] Although not yet formally proven in the chicken, the localization of immune complexes seems to be a prerequisite for germinal centre development as in mammals,[25] as after intravenous injection of preformed immune complexes, germinal centres develop faster and in higher numbers than with the native antigen (unpublished results). Because we were unable to vizualize the preformed IBDV–Icx vaccine shortly after inoculation, the IBDV immune complexes found at later timepoints most probably consist of both newly formed IBDV and specific antibodies. As in IBDV–Icx-vaccinated chickens, most IBDV is localized inside splenic germinal centres from the first moment of detection, this result indicates that undetectable amounts of virus–antibody complex had localized at this site before replication started. In IBDV vaccinated chickens, specific antibodies are produced as well during the infection that theoretically could lead to immune complex formation and subsequent localization in germinal centres. It was previously shown that the levels of specific antibodies induced after vaccination with IBDV and IBDV Icx vaccines were similar for both types of vaccine.[8] The reason for the absence of newly formed immune complexes in IBDV vaccinated chickens may, therefore, be twofold. In these chickens virus replication seems to proceed faster, longer and to a higher amount of virus. Because the levels of antibodies are similar in both vaccination groups,[8] the amount of circulating virus will determine the ratio of IBDV: antibodies and thus whether immune complexes are formed in excess of antigen or excess of antibody. The ratio of IBDV-antibody influences the physicochemical properties of immune complexes. It is conceivable that in IBDV vaccinated chickens immune complexes are formed in excess of antigen that do not localize on (precursor) follicular dendritic cells and thus do not induce germinal centres. On the other hand, it is also possible that the total B-cell depletion has led to a disappearance of FDC and thus that immune complexes find no place to localize, so that germinal centres can not be induced.

With respect to the mechanism of action of this IBDV–Icx vaccine, we conclude that the hypothesis concerning the shielding of vaccine IBDV from mechanisms that lead to reduced antigen load, such as maternal antibodies, is very unlikely. The differences between IBDV and IBDV–Icx were caused by other factors than just the absolute amounts of virus particles. Although both vaccines contained as much IBDV at inoculation, the virus replication was much higher with IBDV vaccine than with the IBDV–Icx vaccine. The remarkable findings that (1) in the bursa of Fabricius small clusters of FDC and B lymphocytes were rescued from depletion, and (2) germinal centres containing IBDV–Icx were induced in the spleen, favour the hypothesis that the IBDV–Icx vaccine exhibits a specific localization in privileged sites of the immune system. The mechanism of action seems therefore related to the ability of the preformed IBDV-containing immune complexes to localize directly on FDC, before detectable virus replication starts.

ACKNOWLEDGMENTS

The authors thank Drs Wim J. A. Boersma (ID-DLO) and Lonneke Vervelde (IAH Compton, UK) for critically reading the manuscript.

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