Research in Veterinary Science 114 (2017) 194–201

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

A recombinant avian antibody against VP2 of infectious bursal disease virus protects chicken from viral infection

Yingjie Zhang[a,b], Jiechao Yin[a], Tianhe Li[c], Bing Zhou[d], Pengfei Xu[a], Ruixiang Che[a], Yunye Liu[a], Hongxue Cao[a], Xianlong Ye[a], Yongbi Yang[a], Xiaole Qi[e], Shimin Zheng[f], Guojie Ding[g], Guiping Ren[a], Hanchun Yang[b,*], Xiaomei Wang[e,**], Deshan Li[a,***]

[a] Biopharmaceutical Lab, College of Life Science, Northeast Agricultural University, Harbin, PR China
[b] Key Laboratory of Animal Epidemiology and Zoonosis of Ministry of Agriculture, College of Veterinary Medicine and State Key Laboratory of Agrobiotechnology, China Agricultural University, Beijing, PR China
[c] University of Chinese Academy of Sciences, Beijing, PR China
[d] Amoy University, Amoy, PR China
[e] Harbin Veterinary Research Institute, Harbin, PR China
[f] College of Veterinary Medicine, Northeast Agricultural University, Harbin, China
[g] Harbin Pharmaceutical Group Bio-vaccine Co., Ltd., Harbin, PR China

ARTICLE INFO

Keywords:
recombinant avian antibody
IBDV
neutralization activity
therapeutic efficacy

ABSTRACT

A stable cell-line was established that expressed the recombinant avian antibody (rAb) against the infectious bursal disease virus (IBDV). rAb exhibited neutralization activity to IBDV-B87 strain in DF1 cells. The minimum rAb concentration required for inhibition of the cytopathic effect (CPE) was 1.563 μg/mL. To test the efficacy of rAb, a 168-h cohabitation challenge experiment was performed to transmit the disease from the chickens challenged with vvIBDV (HLJ0504 strain) to three test groups of chickens, i.e. (1) chickens treated with rAb, (2) chickens treated with yolk antibody, and (3) non-treatment chickens. The survival rates of chickens treated with rAb, yolk antibody and without treatment were 73%, 67% and 20%, respectively. Another batch of chickens was challenged with IBDV (BC6/85 strain) and then injected with rAb (1.0 mg/kg) 6, 24 and 36 h post-challenge. Non-treatment chickens had 100% morbidity, whereas those administered with rAb exhibited only 20% morbidity. Morbidity was evaluated using clinical indicators and bursal histopathological section. This study provides a new approach to treating IBDV and the rAb represents a promising candidate for this IBDV therapy.

1. Introduction

Infectious bursal disease (IBD) is considered to be one of the most important diseases threatening the global poultry industry (Sapats et al., 2003). IBD is caused by infectious bursal disease virus (IBDV), which is a double-strand RNA virus belonging to the *Birnaviridae* family (Berg, 2000; Eterradossi and Saif, 2008). IBDV replicates specifically in developing B-lymphoid cells, resulting in the destruction of the precursors of antibody-producing B cells in the bursal of Fabricius. Consequently, the developing immunosuppression leads to vaccination failures and susceptibility to other infections and diseases (Lukert and Saif, 1997). Because of the prevalence of very virulent IBDV, and treatment of IBDV is not extremely important. Prophylaxis (here vaccination) is indeed extremely important. Treatment is an exception in the poultry industry due to the high costs associated with it. (Malik et al., 2006; Corley and Giambrone, 2002). Vaccination is the most used measure to fight infections with IBDV (Deb et al., 2015). Antibody treatment against IBDV is not widely used. It has been shown experimentally already in the 1970′ that IBD could be prevented by passive immunity (transfer of antibodies from a vaccinated to a non-vaccinated chicken). It might be possible that there are some products in the market (not to the referee's knowledge). But it is not widely used (Lucio and Hitchner, 1980). Therapeutic antibody treatment is currently an effective prophylaxis and treatment of IBDV (Malik et al., 2006).Chicken egg yolk antibody, referred to as immunoglobulin Y (IgY), is now widely used to prevent and treat IBD, especially as it possesses other advantages including cost-effectiveness, convenience and high yield (Carlander et al., 2000). However, using yolk antibodies

* Correspondence to: H. Yang, College of Veterinary Medicine, China Agricultural University, Beijing 100193, PR China.
** Correspondence to: X. Wang, Harbin Veterinary Research Institute, Harbin 150001, PR China
*** Correspondence to: D. Li, College of Life Science, Northeast Agricultural University, Harbin 150030, PR China.
E-mail addresses: yanghanchun1@cau.edu.cn (H. Yang), xmw@hvri.ac.cn (X. Wang), 969670912@qq.com (D. Li).

http://dx.doi.org/10.1016/j.rvsc.2017.04.003
Received 21 September 2016; Received in revised form 8 March 2017; Accepted 7 April 2017

Table 1
Primers used in the amplification of the target genes.

Primers	Primer sequences(5′-3′)
V_HF	GGATCC ACTGGTGCCGTGACGTTGGACGAG(*BamH I*)
V_HR	GCTAGCGGAGGAGACGATGACTTCGGTCC(*Nhe I*)
C_HF	GCTAGCGCGAGCCCCACATCGCCCCCCCGAT(*Nhe I*)
C_HR	GAATTCATTA TTTACCAGCCTGTTTCTGCAGCGTG(*EcoR I*)
V_LF	GGATCCACTGGTGCGCTGACTCAGCCGTCCTCGGTGTC(*BamH I*)
V_LR	TGATGGTGGGGGCCACCTTGGGCTGACCTAGGACGGTCAGGG
C_LF	CGGGACAACCCTGACCGTCCTAGGTCAGCCCAAGGTGGCCCCCACCATCA
C_LR	GCTAGCATTAGCACTCGGACCTCTTCAGGGTCTTC(*Nhe I*)
EGFPR	GAATTCTTACTTGTACAGCTCGTCCATG(*EcoR I*)

Note: F means forward primer, R means reverse primer. Underline is shown as the restriction site of enzyme. The primers were synthesized by Invitrogen (Beijing, China).

in the prophylaxis and treatment of IBDV infection has some shortcomings, such as the potential risk of transmitting the infectious disease and the low concentration of the specific antibody against IBDV in egg yolk (2%–10%) (Mine and Kovacs-Nolan, 2002; Schade et al., 1996).It is therefore imperative to develop new approaches to replace the yolk-derived antibodies for the prophylaxis and treatment of IBD.

Genetic engineering provides strategies for the production of recombinant antibodies. Recently, the field of recombinant antibodies has rapidly progressed because of the interest in their therapeutic use. Recombinant antibodies have many advantages (Sapats et al., 2003). First, there is no concern for the contamination of infectious pathogens during the production process. Second, specific and monoclonal antibodies with high affinity can be obtained by gene manipulation technology. Third, the manufacturing process and quality control procedures can be easily established. Therefore, recombinant antibodies from chickens may be an option to treat IBD. In a previous study (Xu et al., 2014), we constructed highly diverse recombinant antibody libraries from the spleens of immunized chickens, and single chain fragment variable (scFv) antibodies against IBDV were isolated from an antibody library. Finally, one IBDV-specific scFv isolated from the library was able to neutralize the virus in vitro. Although scFv has the advantages of lower immunogenicity and better tissue penetration (Yokota et al., 1992), the half-life reduces *in vivo* because of the lack of an Fc domain in the scFv structure. Moreover, the affinity of an original antibody was sometimes impaired in the corresponding scFv (Jiang et al., 2002; Miyashita et al., 1997). For these reasons, it is preferable to use the full-length antibody which can persist for weeks in the serum compared to the scFv. The objective of this study was to obtain the full-length antibody which against the infectious bursal disease virus.

2. Materials and methods

2.1. Viruses, cells and plasmids

The virus vaccine strains (GT, NF8, 1–65, BJ836, MB and B87) were purchased from the Harbin Pharmaceutical Group Holding Co., Ltd. The virus strain vvIBDV HLJ0504 was maintained in the Harbin Veterinary Research Institute (HVRI) (Chinese Academy of Agricultural Science, China). The virus strain IBDV BC6/85 strain was provided by the QYH Biotech Company Limited. The DF1 cell line, Chinese hamster ovary (CHO-K1-SV) cell line, Pkappa vector and pBSD-scFv plasmid (containing V_H and V_L gene fragments of the anti-IBDV neutralization antibody) were laboratory stock (Xu et al., 2014). Pee6.4 and Pee12.4 eukaryotic expression plasmids (containing glutamine synthetase (GS) gene, a selectable marker) were purchased from Lonza, Switzerland.

2.2. Antibodies and antigen

The HRP-goat anti-chicken antibody was purchased from eBioscienc. The anti-IBDV egg yolk antibody was purchased from Pulike Biological Engineering, INC. The VP2 protein was expressed in *E. coli* (unpublished data) and purified by an AKTA Purifer 100 (GE, USA).

2.3. Primers

2.4. Animals

One-hundred and thirty (130), 21-day-old specific pathogen free (SPF) chickens were purchased from the HVRI and housed in negative-pressure-filtered air isolators. Animal experiments were approved by the Animal Ethics Committee of the Institute.

2.5. Reagents

DNA markers and restriction enzymes were purchased from TaKaRa. Protein marker was purchased from Fermentas. Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco. Serum free media was purchased from WISENT, INC.

2.6. Construction of plasmid which contains genes of recombinant avian antibody

The V_L (Variable region of light chain) and V_H (Variable region of heavy chain) genes of the recombinant avian antibody were amplified by PCR from plasmid pBSD-scFv with two pairs of primers (V_LF, V_LR and V_HF, V_HR). The C_L (Constant region of light chain) gene of the recombinant avian antibody was synthesized according to the sequences published in GenBank (accession numbers of K00678) and confirmed by sequencing. The C_H (Constant region of heavy chain) gene of recombinant avian antibody was synthesized according to the sequences published in GenBank (accession numbers of × 07174) and confirmed by sequencing. The C_L gene and C_H gene were synthesized in Invitrogen (Beijing, China). Using enzyme digestion and ligation, the recombinant expression vector was successfully constructed and named Peedual-IRES (Fig. 1E). The strategy for the construction of rAb was showd as below (Fig. 1).

2.7. Screening the cell-line exhibiting high expression of recombinant avian antibody

The Peedual-IRES plasmid containing the genes of rAb was trans-

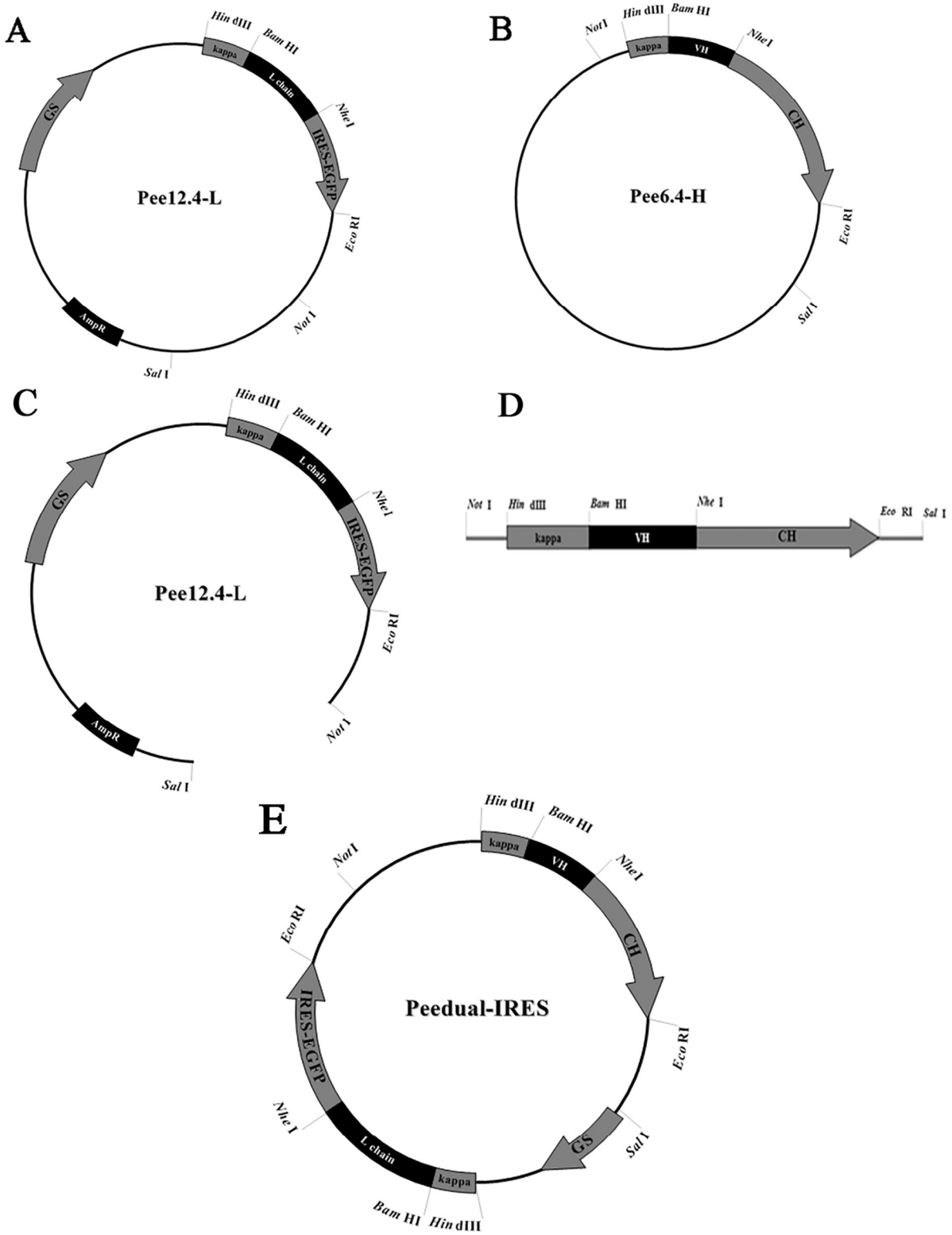

Fig. 1. The schematic diagram of Peedual-IRES vector construction. A The gene of the L chain containing the kappaleader signal sequence and the gene of the IRES-EGFP fragment were inserted into the Pee12.4 vector; B.The gene of the H chain containing the kappaleader signal sequence was inserted into the Pee6.4 vector; C The Pee12.4 vector was digested with enzymes "*Sal* I, *Not* I"; D The Pee6.4 vector was digested with enzymes "*Sal* I, *Not* I"; E Fragments which contained the L and H genes of the recombinant avian antibody were ligated and the recombinant expression vector named Peedual-IRES.

fected into CHO-K1-SV cells. As the plasmid contained the glutamine synthetase (GS, GS is the enzyme responsible for the biosynthesis of glutamine using glutamate and ammonia as substrates) gene and the EGFP gene (as the selectable markers), these two selectable markers were used to screen the cell-line exhibiting a high expression of rAb. The cells were cultured in selective medium (containing methionine sulphoximine (MSX) which selectively inhibits the activity of the GS gene) for 3 weeks, thereby killing most of the artificial GS-gene negative cells. The surviving cells were observed for their fluorescence intensity because of the EGFP, using a fluorescence microscope.

Fluorescence intensity among the monoclonal cell lines is not homogeneous, and stronger fluorescence intensity indicates a stronger ability of cells to express exogenous genes. Therefore, flow cytometer methods (FCM) were used to further screen the cells exhibiting a high expression of rAb.

2.8. The expression of recombinant avian antibody

Cells exhibiting a high expression of rAb were cultured in serum-free medium. The supernatant was collected every three days and the supernatant centrifuged and condensed by a hollow fiber column (GE, USA) (HFC).

2.9. Western blotting

The VP2 protein was subjected to SDS-PAGE, and transferred onto a nitrocellulose membrane. The membrane was blocked with 5% (w/v) skimmed milk in PBS buffer. The membrane was incubated with 100 μL rAb (40 μg/mL) in PBS at 4 °C overnight, then incubated with HRP-goat anti-chicken antibody (1:7500) for 1 h at 37 °C. The membrane was developed using an ECL detection system.

2.10. Relative specifically analysis of recombinant avian antibody

To measured a concentration dependent reactivity (ability to be detected) of the rAb with a given amount of VP2, 96-well immunoassay plates were coated with VP2 protein (40 μg/mL) overnight at 4 °C. The wells were blocked with TBS blocking buffer for 2 h at 37 °C (250 μL/well), and 100 μL rAb at several concentrations (400, 80, 16 and 3.2 μg/mL) was added into the wells in triplicate. The 96-well plates were incubated at 37 °C for 1 h then washed with TBST followed by incubation with HRP-goat anti-chicken antibody (1:7500) in TBS blocking buffer. Three negative controls were included: Negative 1 (the supernatant of the serum-free medium culturing CHO-K1-SV cells with no transfection), Negative 2 (without rAb) and Negative 3 (without HRP-goat anti-chicken antibody).

To qualitative assay (positive/negative) between rAb and different IBDV vaccine strains, 96-well immunoassay plates were coated with different IBDV vaccine strains ($100TCID_{50}$) overnight at 4 °C. Wells were blocked with TBS blocking buffer for 2 h at 37 °C (250 μL/well). 100 μL/well of rAb (400 μg/mL) was added into the wells in triplicate, then incubated at 37 °C for 1 h. Plates were washed with TBST and incubated with HRP-goat anti-chicken antibody (1:7500) in TBS blocking buffer at 37 °C for 1 h. Two negative controls were included: Negative 1 (the supernatant of the serum-free medium culturing CHO-K1-SV cells with no transfection), and Negative 2 (Newcastle Disease Virus (NDV)). Two assays were developed using TMB solution. The development of color product was terminated by 50 μL of 0.1 mol/LH_2SO_4. The absorbance of each well was measured with an ELISA reader (Bio−Rad type 680) at 450 nm.

2.11. The titer of IBDV

The chicken embryo fibroblasts cells (DF1 cells) were cultured in DMEM medium containing 10% FBS. IBDV, derived by passaging of IBDV vaccinate (B87 strain, 100 μL) in chicken embryo, was diluted with DMEM. The logarithmic phase DF1 cells were transferred to flat-bottom, 96-well plates (100 μL in each well) and the monolayer DF1 cells treated with Log_2 dilution of IBDV (100 μL in each well), then incubated at 37 °C in the presence of 5% CO_2. The cell monolayers were examined visually for a cytopathic effect after 7d. The cells of the control were treated in the same way with 100 μL DMEM. Samples were measured in eight replicates and each experiment was repeated at least twice. The 50% tissue culture infective dose ($TCID_{50}$) of virus was calculated by the Reed-Muench method (Reed and Muench, 1938).

2.12. The recombinant avian antibody neutralization test

rAb (100 μL) with different concentrations (400 to 0.782 μg/mL) were incubated with 100 $TCID_{50}$ of IBDV (100 μL) at 37 °C for 1 h. The rAb-virus mixture was then incubated with the DF1 monolayers in 96-well plates at 37 °C in the presence of 5% CO_2. The positive control group was treated with IBDV (100 $TCID_{50}$, 100 μL) and DMEM (100 μL). The negative control group was treated with DMEM (200 μL). A virus back-titration was performed to assess the actual virus titer used in experiment. CPE was observed using an inverted microscope on day 7 post-inoculation, and the samples measured in eight replicates.

2.13. The half-life of the recombinant avian antibody in vivo

Thirty (30) 21-one-day-old SPF chickens were randomly divided into three groups, i.e., rAb, yolk antibody (yAb), and the saline group, each with 10 chickens. The chickens were injected with 1.0 mg/kg rAb and yAb intramuscularly. On Days 1, 4, 7, 10, 13 and 16 post-injection, 1.0 mL of blood was collected from each chicken and ELISA used to measure the contents of rAb and yAb in the serum. Briefly, 96-well immunoassay plates were coated with VP2 protein (40 μg/mL) overnight at 4 °C. The wells were blocked with TBS blocking buffer for 2 h at 37 °C (250 μL/well), and the serum of PBS group, yolk antibody group and rAb group collected in different time-phase was added into the wells in triplicate, then incubation with HRP-goat anti-chicken antibody (1:7500) in TBS blocking buffer. The serum collected on day 0 was used as negative control. The assay was developed using TMB solution. The development of color product was terminated by 50 μL of 0.1 mol/LH_2SO_4. The absorbance of each well was measured with an ELISA reader (Bio−Rad type 680) at 450 nm.

2.14. The cohabitation challenge experiment in chickens

A total of 60, 21-one-day-old SPF chickens were randomly divided into four groups, each with 15 chickens, i.e. the model control group (challenged with HLJ0504 strains with 0.2×10^3 ELD_{50}), the rAb treatment group (injected with 1.0 mg/kg rAb intramuscularly), the yolk antibody (yAb) treatment group (injected with 1.0 mg/kg yAb intramuscularly) and normal control (injected with PBS intramuscularly). Chickens of the model control group were challenged with IBDV, and chickens in the other groups were injected with rAb, yAb and PBS, respectively. All of the chickens were housed together for 168 h, after which the survival rate of each group was calculated.

2.15. The treatment experiment in chickens with IBD

Forty (40), 21-one-day-old SPF chickens were randomly divided into four groups each with 10 chickens, i.e. the model control group, rAb treatment group, yolk antibody (yAb) treatment group and normal control group (unchallenged and non-treatment). The chickens of each group were housed in separate negative-pressure-isolators. During the experiment, chickens of the model control group, rAb treatment group and yAb treatment group were challenged with BC6/85 strain with 0.2×10^4 BID_{50} at 21-days-of-age. The rAb and yAb chickens were injected 6, 24 and 36 h post-challenge (1.0 mg/kg intramuscularly) then sacrificed 72 h post-challenge. Bursale were weighed and the bursall/body weight ratio (BF/BW) calculated. Bursal lesion scores were given to each chicken based on the increasing severity of bursall atrophy (0 = no lesion, 1 = slight change, 2 = scattered or partial bursall damage, 3 = 50% or less follicle damage, 4 = 51–75% follicle damage, 5 = 76–100% bursall damage) (Li et al., 2013; Shaw and Davison, 2000).A chicken with a bursall lesion score of 1 and a BF/BW ratio of not < 2 standard deviations (S.D.) below the average ratio of the normal control group was defined as having been protected against the IBDV challenge.

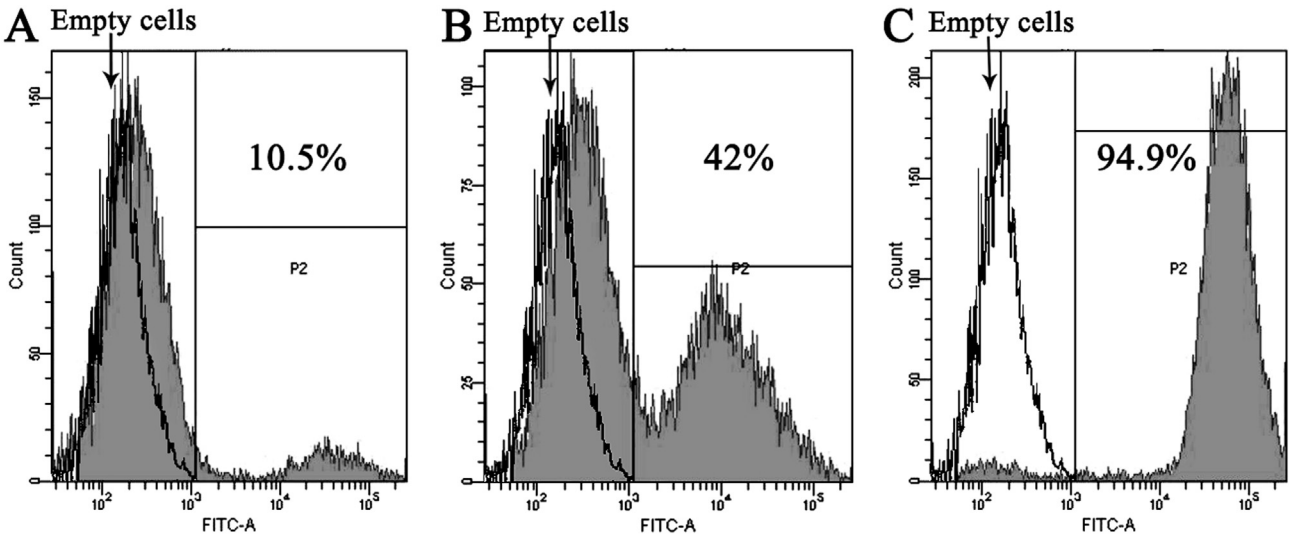

Fig. 2. The cells with high expression of recombinant avian antibody were obtained by the screening of flow cytometer. The solid peaks represented rAb-transfected cells which expressed EGFP. The hollow peaks represented cells without transfecting with recombinant plasmid which were used as negative controls. (A) The first round of screening. (B) The second round of screening. (C) The third round of screening.

2.16. Statistical analysis

All experimental data were analyzed by one-way analysis of variance (ANOVA) followed by the Students two-tailed *t*-test using SPSS13.0 software. $P < 0.05$ was deemed to indicate statistical significance of differences.

3. Results

3.1. Plasmid of recombinant avian antibody construction

The correct sequences of the L and H chain contained by the recombinant expression vector (Peedual-IRES) were confirmed by DNA sequencing (data not show).

3.2. Screening of the cells with high expression of recombinant avian antibody

Non-recombinant cells were killed by culturing in selective medium. Also, cells with high expression of EGFP were selected by flow cytometric analysis. The fluorescence intensity increased to 10.5, 42.0 and 94.9% after the first, second and third round of screening, respectively (Fig.2).

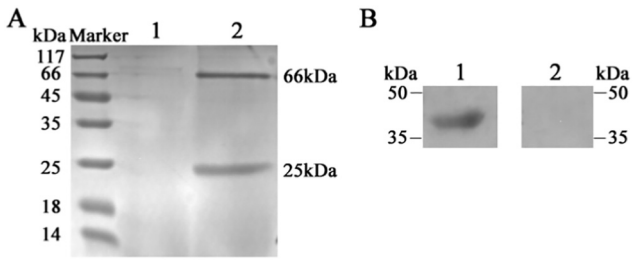

Fig. 3. The expression and identification of recombinant avian antibody. SDS-PAGE was used to analyse rAb in the supernatant; VP2 protein was subjected to SDS-PAGE and transferred onto membrane, then the membrane was incubated with the supernatant of the serum-free medium which cultured CHO-K1-SV cells transfected with or without the eukaryotic expression plasmid (Peedual-IRES), followed by incubation with HRP-goat anti-chicken antibody. (A) The expression of recombinant avian antibody; 1 The supernatant of the serum-free medium which cultured CHO-K1-SV cells with no transfection; 2 The supernatant of the serum-free medium which cultured CHO-K1-SV cells transfected with the eukaryotic expression plasmid (Peedual-IRES). (B) The identification of recombinant avian antibody by western blotting. 1 Incubation with the supernatant of the serum-free medium which cultured CHO-K1-SV cells transfected with the eukaryotic expression plasmid; 2 Incubation with the supernatant of the serum-free medium which cultured CHO-K1-SV cells without the eukaryotic expression plasmid.

3.3. The expression and identification of recombinant avian antibody

SDS-PAGE analysis showed that the cells expressed the H chain protein of rAb (approximately 66 kDa), and the L chain protein of rAb (approximately 25 kDa). The rAb reacted with VP2 protein specifically (Fig. 3A, B).

3.4. The recombinant avian antibody titers to VP2 or different IBDV vaccine strains by ELISA

The ELISA showed that an antibody concentration of 3.2 μg/mL was still able to detected 40 μg/mL VP2 bound to the plate and the rAb can react to different IBDV vaccine strains bound to the plate compared with Negative 1 (Fig. 4).

3.5. The titer of IBDV

The numbers of DF1 cells with cytopathic effect (CPE) were showed as blew (Table 2), the 50% tissue culture infective dose ($TCID_{50}$) of the virus was calculated by the Reed-Muench method, $TCID_{50} = 10^{6.4}/0.1$ mL.

3.6. The neutralization activity of recombinant avian antibody to IBDV B87 strain

The rAb exhibited a high ability to neutralize 100 $TCID_{50}$ of IBDV. The minimum concentration of rAb, which completely suppressed CPE induced by the virus in 8- of 8-wells, was 1.563 μg/mL (Table 3).

3.7. The half-life of recombinant avian antibody in vivo

The results showed that there was no significant difference between the half-life of rAb and yAb (Fig. 5).

3.8. Survival rates in chickens challenged with vvIBDV

In the cohabitation challenge experiment, the survival rates of chickens at 72 h post-challenge in the model control, rAb treatment group, yAb treatment group and normal control were 93.3, 100, 100

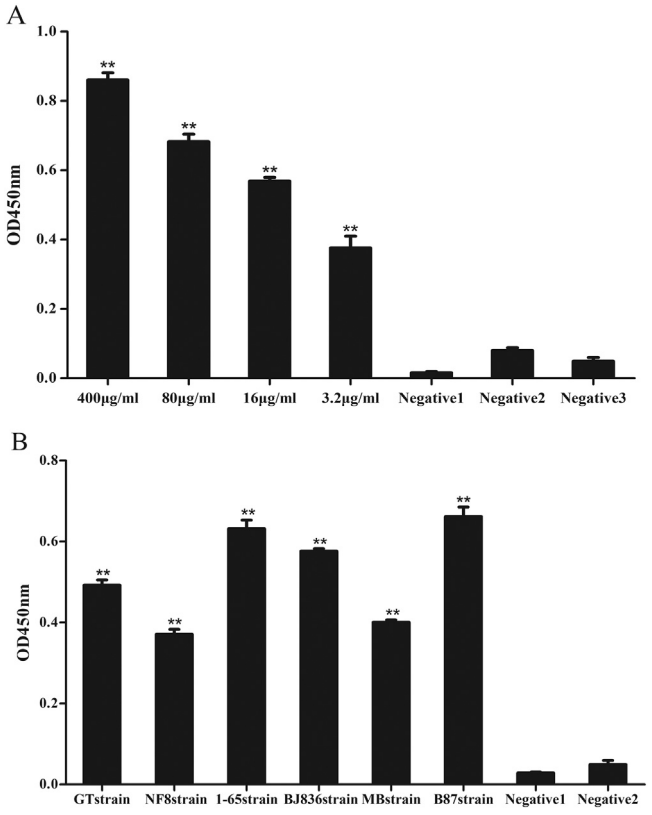

Fig. 4. The recombinant avian antibody react to VP2 or different IBDV vaccine strains. The plates were coated with VP2 protein or different IBDV vaccine strains, followed by incubating with the supernatant of the serum-free medium which cultured CHO-K1-SV cells transfected with the eukaryotic expression plasmid (Peedual-IRES). The absorbance of each well was measured with ELISA reader at UV wave length of 450 nm. The data are the means ± S.D. of quadruplex samples. (A) The specifically of rAb (400 μg/mL, 80 μg/mL, 16 μg/mL, 3.2 μg/mL) to VP2 protein. (B) The specifically of rAb to different IBDV vaccine strains.Note: $^{**}P < 0.01$ vs Negative 1.

Table 2
The numbers of wells with cytopathic effect (CPE).

Dilution of virus	The number of CPE	The number of non-CPE
10^1	8	0
10^2	8	0
10^3	8	0
10^4	8	0
10^5	8	0
10^6	5	3
10^7	2	6
10^8	0	8
10^9	0	8
10^{10}	0	8
10^{11}	0	8

The IBDV (B87 strains) was diluted by DMEM. The samples were measured in 8 replicates, data were obtained from three independent experiments.

Table 3
The neutralization activity of recombinant avian antibody to IBDV (B87 strain).

Concentration of rAb(μg/mL)	The cell with CPE or non-CPE		
	rAb	DMEM	IBDV
400	−	−	+
200	−	−	+
100	−	−	+
50	−	−	+
25	−	−	+
12.5	−	−	+
6.25	−	−	+
3.125	−	−	+
1.563	−	−	+
0.782	+	−	+

+: Cytopathic effects observed, − No cytopathic effects detected. The samples were measured in 8 replicates, and data were obtained from three independent experiments.

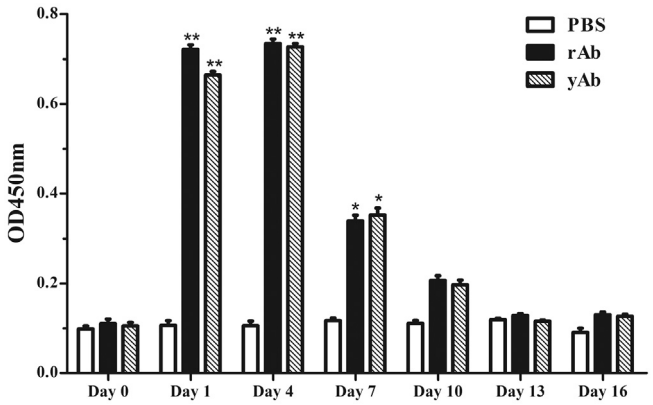

Fig. 5. The half-life of recombinant avian antibody *in vivo*. 30 twenty-one-day-old SPF chickens were randomly divided into 3 groups: rAb group, yolk antibody (yAb) group and saline group. Each group contained 10 chickens. The chickens were injected with rAb and yolk antibody intramuscularly at a dose of 1 mg/kg. Blood was drawn from each chicken on day 0, day 1, day 4, day 7, day 10, day 13 and day 16 after injection. The blood was centrifuged to obtain serum. ELISA was used to measure the contents of rAb and yolk antibody.Note: $^{*}P < 0.05$ vs the Day 0 ($n = 3$), $^{**}P < 0.01$ vs the Day 0($n = 3$).

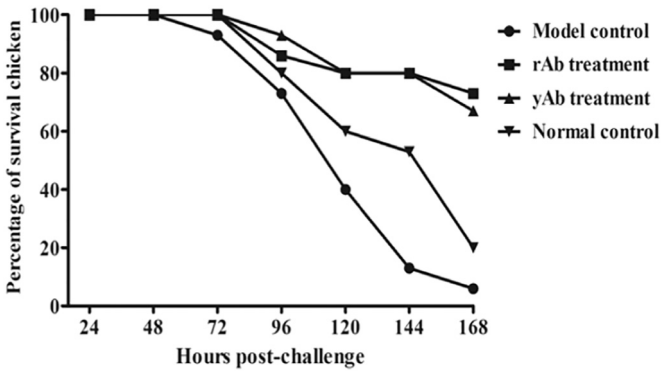

Fig. 6. The survial rates in the cohabitation challenge experiment. In this experiment, chickens of model control group were challenged with vvIBDV (HLJ0504 strain), and chickens in other groups were injected with rAb, yAb and PBS simultaneously, respectively. Then all of chickens cohabitated for 168 h, and the survival rates of each group were caculated.

and 100%, respectively, at 96 h post-challenge 73.3, 86.7, 93.3 and 80%, respectively, at 120 h 40, 80, 80 and 60%, respectively, at 144 h post-challenge 13.3, 80, 80 and 53.3% respectively, and at 168 h post-challenge 6, 73, 67 and 20%, respectively (Fig. 6).

3.9. Protection against IBDV challenge

The BF/BW ratios, bursal lesion scores and protection rate are presented in Table 4. Chickens in the normal control group remained healthy and had normal sized bursale. After the IBDV challenge, the BF/BW ratio in the model control group (2.62 ± 0.34) decreased significantly compared to the normal control group (5.72 ± 0.44); BF/BW ratio in the rAb treatment group was 4.65 ± 0.43, which was increased significantly compared to the model control group and the yAb treatment group (3.29 ± 0.21). Chickens in the model control group exhibited typical clinical signs of the disease and a 100% infection rate. The protection rate in the rAb treatment group was

Table 4
Protection efficacy against IBDV (BC6/85 strain) challenge of each group in chickens.

Groups[a]	BF/BW ratio[b]	Histopathological BF lesion scores[c]						Protection[d](%)
		0	1	2	3	4	5	
Model control	2.62 ± 0.34$^{\#\#}$	0	0	0	0	3	7	0/10 (00.0)
rAb treatment	4.85 ± 0.43$^{*\#}$	2	6	1	0	1	0	8/10 (80.0)
yAb treatment	3.29 ± 0.21$^{\#}$	0	4	2	1	3	0	4/10 (40.0)
Normal control	5.72 ± 0.44	10	0	0	0	0	0	10/10 (100.0)

[a] Chickens were treatment with recombinant antibody (rAb) and yolk antibody (yAb). In addition, challenged (model control), and unchallenged (normal control) chickens were kept for controls.

[b] BF/BW ratios was calculated by bursall weight × 1000 then divided by body weight and presented as the mean ± standard deviation from each group. Values followed by the same letter within the column do not differ significantly ($P > 0.05$) as determined by one-way ANOVA. $^{*}p < 0.05$ compared with model control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with normal control.

[c] Histological bursal lesion scores were given to each chicken based on the increasing severity of bursall atrophy (0: no lesion, 1: slight change, 2: scattered or partial bursall damage, 3: 50% or less follicle damage, 4: 51–75% follicle damage, 5: 76–100% bursall damage).

[d] Protection was defined by the number of chickens with histopathological BF lesion score 0 and 1/the number of chickens in the group.

80%, whereas the protection rate in the yAb treatment group was only 40%. Together, these data showed that the rAb had a good efficacy in treating IBD.

4. Discussion

Infectious bursal disease virus is a pathogen of major economic importance in the poultry industry. In this study, we use the genetic engineering technology to create recombinant antibody against IBDV. As we know, the research and development of therapeutic antibodies is a rapidly progressing field (Aggarwal, 2009; Beck et al., 2010).In the past 30 years, > 30 immunoglobulins (IgGs) and their derivatives have been approved for use, such as infliximab, rituximab and trastuzumab (Reichert, 2009a; Reichert, 2010). The antibody research has hinted at the promise of new versatile therapeutic agents to fight cancer, autoimmune diseases and infection. Technology development and the testing of new generations of antibody reagents have altered our view of how they might be used for prophylactic and therapeutic purposes. The therapeutic antibodies of today are genetically engineered molecules that are designed to ensure high specificity and functionality (Brekke and Sandlie, 2003). The use of biotechnology to create therapeutic antibodies holds many advantages for the future. Phage-display and bacterial-display technology are methods used for screening recombinant antibodies from antibody libraries. Currently, phage-display technology is the major method for screening recombinant antibodies from antibody libraries. However, phage-display is time consuming, taking at least one year to obtain the desired antibodies. In contrast, bacteria-display technology offers an efficient way to process library screening with FCM to diminish the screening time and enable real-time visualization to identify desired antibody clones (Dane et al., 2006; Jung et al., 2007).

Therapeutic antibodies for human diseases like cancers, autoimmune diseases and virus infections are most frequently studied. However, genetically engineered therapeutic antibodies for animal diseases have not been reported. The present study describes a new method that uses bacterial display isolates, scFv antibodies, against IBDV from an antibody library via the VP2 protein (Xu et al., 2014). The VP2 protein is a component of IBDV (Mundt et al., 1995). It has been reported that the VP2 protein is the major protective antigen of the virus and contains antigenic epitopes responsible for the induction of neutralization antibodies (Becht et al., 1988). By using bacterial-display, we obtained the scFv antibody with neutralization activity. Based on the sequence of scFv antibody with neutralization activity, a recombinant eukaryotic expression plasmid with double cistrons containing the sequence of the rAb, was constructed. In addition, the recombinant plasmid also contained the EGFP gene. FCM was used to screen those cells exhibiting high expression of the rAb as determined by EGFP. The CHO-K1SV cell line was chosen to produce the rAb. The stable cell-line exhibiting high expression of the rAb was obtained after three rounds of FCM screening. Finally, a eukaryotic expression system that can be used for the rapid screening of eukaryotic cell lines expressing recombinant avian antibody was developed.

Condensing by a hollow fiber column obtained a high concentration of rAb. The relative affinity assay showed that the rAb could specifically react with VP2 protein of the IBDV virulent strain and different IBDV vaccine strains including the GT, NF8, 1–65, BJ836, MB and B87 strains. Moreover, the rAb exhibited high neutralization activity to IBDV-B87 with a minimum concentration for the inhibition of CPE of 1.563 μg/mL. These results suggest that rAb has the potential to treat IBD. The half-life of rAb in the serum was approximately seven days. A cohabitation challenge experiment showed that administration of rAb increased the survival rate compared to chickens without. Furthermore, administration of rAb exhibited good efficacy and a high protection rate (80%) compared to challenge chickens without treatment, and challenged chickens treated with yAb (their protection rate was only 40%). These data indicate that rAb has the therapeutic efficacy to treat IBD.

In conclusion, a eukaryotic expression system was established which was used for the rapid screening of a eukaryotic cell-line expressing recombinant avian antibody. The recombinant avian antibody exhibited high neutralization activity and good therapeutic efficacy, which indicate that the recombinant avian antibody has the potential to be a new therapeutic antibody in the treatment of IBD.

Acknowledgement

This research was supported by Heilongjiang province project of applied technology research and development (2013GC13C105) and The National Natural Science Fund biologic science base improve program of research training and capacity (J1210069/J0124).

References

Aggarwal, S., 2009. What's fueling the biotech engine-2008. Nat. Biotechnol. 27 (11), 987–993.
Becht, H., Müller, H., Müller, H.K., 1988. Comparative studies on structural and antigenic properties of two serotypes of infectious bursal disease virus. J. Gen. Virol. 69 (3), 631–640.
Beck, A., Reichert, J.M., Wurch, T., 2010, March. 5th European antibody congress 2009: November 30–December 2, 2009, Geneva, Switzerland. In: MAbs. Taylor & Francis (Vol. 2, No. 2, pp. 108–128).
Berg, T.P.V.D., 2000. Acute infectious bursal disease in poultry: a review. Avian pathology 29 (3), 175–194.
Brekke, O.H., Sandlie, I., 2003. Therapeutic antibodies for human diseases at the dawn of the twenty-first century. Nat. Rev. Drug Discov. 2 (1), 52–62.
Carlander, D., Kollberg, H., Wejåker, P.E., Larsson, A., 2000. Peroral immunotheraphy with yolk antibodies for the prevention and treatment of enteric infections. Immunol. Res. 21 (1), 1–6.
Corley, M.M., Giambrone, J.J., 2002. Immunosuppression in specific-pathogen-free broilers administered infectious bursal disease virus vaccines by in ovo route. Avian Dis. 46 (4), 810–815.
Dane, K.Y., Chan, L.A., Rice, J.J., Daugherty, P.S., 2006. Isolation of cell specific peptide ligands using fluorescent bacterial display libraries. J. Immunol. Methods 309 (1), 120–129.

Deb, R., Dey, S., Mohan, C.M., Gaikwad, S., Kamble, N., Khulape, S.A., Gupta, S.K., Maity, H.K., Pathak, D.C., 2015. Development and evaluation of a salmonella typhimurium flagellin based chimeric DNA vaccine against infectious bursal disease of poultry. Res. Vet. Sci. 102, 7–14.

Eterradossi, N., Saif, Y.M., 2008. Infectious bursal disease. Diseases of Poultry 12, 185–208.

Jiang, X., Ookubo, Y., Fujii, I., Nakano, H., Yamane, T., 2002. Expression of fab fragment of catalytic antibody 6D9 in an *Escherichia coli* in vitro coupled transcription/translation system. FEBS Lett. 514 (2–3), 290–294.

Jung, S.T., Jeong, K.J., Iverson, B.L., Georgiou, G., 2007. Binding and enrichment of *Escherichia coli* spheroplasts expressing inner membrane tethered scFv antibodies on surface immobilized antigens. Biotechnol. Bioeng. 98 (1), 39–47.

Li, K., Gao, H., Gao, L., Qi, X., Gao, Y., Qin, L., Wang, Y., Wang, X., 2013. Adjuvant effects of interleukin-18 in DNA vaccination against infectious bursal disease virus in chickens. Vaccine 31 (14), 1799–1805.

Lucio, B., Hitchner, S.B., 1980. Immunosuppression and active response induced by infectious bursal disease virus in chickens with passive antibodies. Avian Dis. 189–196.

Lukert, P.D., Saif, Y.M., 1997. Infectious Bursal disease. In: Calnek, B.W. (Ed.), Diseases of Poultry, 10th ed. Iowa State University Press, Ames, pp. 721–738.

Malik, M.W., Ayub, N., Qureshi, I.Z., 2006. Passive immunization using purified IgYs against infectious bursal disease of chickens in Pakistan. J. Vet. Sci. 7 (1), 43–46.

Mine, Y., Kovacs-Nolan, J., 2002. Chicken egg yolk antibodies as therapeutics in enteric infectious disease: a review. J. Med. Food 5 (3), 159–169.

Miyashita, H., Hara, T., Tanimura, R., Fukuyama, S., Cagnon, C., Kohara, A., Fujii, I., 1997. Site-directed mutagenesis of active site contact residues in a hydrolytic abzyme: evidence for an essential histidine involved in transition state stabilization. J. Mol. Biol. 267 (5), 1247–1257.

Mundt, E., Beyer, J., Müller, H., 1995. Identification of a novel viral protein in infectious bursal disease virus-infected cells. J. Gen. Virol. 76 (2), 437–443.

Reichert, J.M., 2009a. Global antibody development trends. MAbs 1 (1), 86–87 (January/February).

Reed, L.J., Muench, H., 1938. A simple method of estimating fifty per cent endpoints. Am. J. Epidemiol. 27 (3), 493–497.

Reichert, J.M., 2010. Antibodies to watch in 2010. Mabs 2:84-100. MAbs 2 (1), 84–100.

Sapats, S.I., Heine, H.G., Trinidad, L., Gould, G.J., Foord, A.J., Doolan, S.G., Prowse, S., Ignjatovic, J., 2003. Generation of chicken single chain antibody variable fragments (scFv) that differentiate and neutralize infectious bursal disease virus (IBDV). Arch. Virol. 148 (3), 497–515.

Schade, R., Staak, C., Hendriksen, C., Erhard, M., Hugl, H., Koch, G., Larsson, A., Pollmann, W., Regenmortel, M., Rijke, E., Spielmann, H., Steinbusch, H., Straughan, D., 1996. The production of avian (egg yolk) antibodies: IgY. ATLA, Nottingham 24, 925–934.

Shaw, I., Davison, T.F., 2000. Protection from IBDV-induced bursal damage by a recombinant fowlpox vaccine, fpIBD1, is dependent on the titre of challenge virus and chicken genotype. Vaccine 18 (28), 3230–3241.

Xu, L.M., Li, T.H., Zhou, B., Guo, M., Liu, M., Zhao, J.Z., Gao, H.W., Li, D.S., 2014. scFv antibodies against infectious bursal disease virus isolated from a combinatorial antibody library by flow cytometry. Biotechnol. Lett. 36 (5), 1029–1035.

Yokota, T., Milenic, D.E., Whitlow, M., Schlom, J., 1992. Rapid tumor penetration of a single-chain Fv and comparison with other immunoglobulin forms. Cancer Res. 52 (12), 3402–3408.