Chicken Monoclonal Antibodies against Synthetic Bovine Prion Protein Peptide

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ABSTRACT. Chicken monoclonal antibodies (mAbs) were developed against bovine prion protein (PrP) peptide. Chickens immunized with bovine PrP peptide B204 (amino acid residues 204–220) coupled to keyhole limpet hemocyanin produced specific antibodies to the peptide as determined by an enzyme-linked immunosorbent assay (ELISA) using the B204 peptide coupled to ovalbumin as target antigen. From a fusion experiment using the chicken fusion partner cell line MuH1 and immune spleen cells, 19 mAbs reactive with B204 were generated. These mAbs were subdivided into five groups based on competitive ELISA using B204 and four 10-amino acid peptides. These five groups included all combinations expected based on comparison of amino acid sequences among the five species, bovine, mouse, human, sheep and hamster, examined. These results indicate that the chicken mAb system is a suitable technique for immunological analysis of PrP in mammals. — **KEY WORDS:** bovine PrP, chicken, monoclonal antibody.

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Scrapie is a naturally occurring transmissible neurodegenerative disease in sheep and goats and is the prototype for all transmissible spongiform encephalopathies (TSE), including transmissible mink encephalopathy in mink, bovine spongiform encephalopathy (BSE) in domestic cattle, Creutzfeldt-Jakob disease (CJD), Kuru and Gerstmann-Sträussler syndrome in humans [15]. TSE are characterized by accumulation of an abnormal form of a cell surface sialoglycoprotein called prion protein (PrP) [4]. The abnormal form of PrP is partially proteinase resistant (PrP^{Sc}) whereas normal cellular PrP isoform (PrP^C) can be completely degraded by proteinase K.

The diagnosis of TSE is usually based on clinical symptoms, the course of the illness, and histopathology. The PrP^{Sc} or CJD PrP isoform (PrP^{CJD}) are also useful as diagnostic markers, using specific polyclonal and monoclonal antibodies (mAbs) raised in mammals [2, 5, 6, 9–11, 19, 20]. However, the higher homology (more than 95%) of mammalian PrP amino acid sequences [14, 16, 18] indicates that the specific epitopes are restricted. Numerous PrP-specific polyclonal antibodies have been raised in rabbits [8–10, 17, 20] as well as a limited number of mouse mAbs directed to PrP [2, 6, 8, 19]. The lower homology (less than 40%) between avian and mammalian PrP suggested that birds might be efficient for the generation of specific antibodies to mammalian PrP.

In our previous studies, we have established many kinds of chicken mAbs [1, 8, 11–13]. The chicken fusion partner cell lines have been improved to generate chicken hybridomas more efficiently [11, 13]. To produce chicken mAbs specific for mammalian PrP in immunological

diagnosis of TSE, in this study, we used bovine PrP synthetic peptide as immunogen and a thymidine kinase-deficient and ouabain resistant chicken B cell line, MuH1 [11], for cell fusion. We describe here the generation of 19 chicken mAbs raised against bovine PrP peptide and characterize their specificity.

Five synthetic PrP peptides were used in this study (Fig. 1). The synthetic bovine PrP peptide B204 (amino acid residues 204–220) was used as immunogen and as antigen for antibody determination. An additional cysteine residue was added at the N-terminal to conjugate the peptide to the carrier proteins keyhole limpet hemocyanin (KLH) and ovalbumin (OA). The B204-KLH was used as immunogen for chickens and the B204-OA was used as antigen for determination of specific antibody by an enzyme-linked immunosorbent assay (ELISA). The other four synthetic PrP peptides used were two bovine peptides B204' (amino acid residues 204–213) and B211 (amino acid residues 211–220), the M211 peptide (amino acid residues 211–220) which is conserved in mouse and human and the S211 peptide (amino acid residues 211–220) which is conserved in sheep and hamster; these were used to determine specificity in a competitive ELISA.

Fig. 1. Comparison of PrP amino acid sequences (amino acid residues 204–220) among chicken, ox, mouse and human, and sheep and hamster. Amino acid sequences of five synthetic peptides, B204, B204', B211, M211 and S211, respectively, used in this study were shown.

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Four one-month-old HB-15 inbred chickens (from Dr. O. Vainio, Turku University) were immunized intramuscularly with 200 µg B204-KLH in 0.5 ml phosphate buffered saline (PBS) together with an equal volume of alum solution. Thirty days later, they received the same antigen without alum three times at 3-week intervals, by the intravenous route. All immunized chickens produced specific antibody to the B204 peptide and their serum antibody titers were each more than 1:15,000. The MuH1 cells were hybridized with the immune spleen cells from one of them three days after the final injection of antigen by the method described previously [11]. From cultures of twenty 96-well plates, 200 wells showed growth and finally 19 cloned hybridomas producing antibodies against the B204 peptide were identified. The chicken mAbs obtained in this study were all classified as IgG (IgY).

The competitive ELISA was performed by using five peptides, B204, B204', B211, M211 and S211 (Table 1). The reactivity of 19 mAbs to the B204 peptide were inhibited with B204 peptide. That of three (A4, E11 and E12, Group A) of these mAbs was also inhibited by B204' peptide but not by the other three peptides, B211, M211 and S211. Of the remaining 16 mAbs which were not inhibited by B204', two (B23 and B24, Group B) were inhibited by three types of peptides B211, M211 and S211, one (E13, Group C) was inhibited by B211 and M211, three (B10, B12 and C5, Group D) were inhibited by B211 and S211, and ten (A2, A3, A6, B5, B9, C3, C4, D21, E18 and E20, Group E) were inhibited only by B211. Therefore, the 19 mAbs raised against B204 peptide were subdivided into five groups based upon the above competitive assay.

Specific antibodies against mammalian PrP have been raised mainly in rabbits [8–10, 17, 20]. Mouse mAbs

directed to PrP [2, 5, 6, 19] are fewer due to higher homology (more than 95%) of PrP genes between mouse and the other mammalian species. Recently, the phage display mouse mAbs directed to PrP have been raised using mice in which the PrP gene was ablated (*Prnp*^{0/0}) [19]. On the other hand, our establishment of chicken mAbs was successful probably due to lower homology (less than 40%) between chicken PrP and mammalian PrP. All chickens immunized with B-204 peptide produced specific antibody and we generated a total of 19 chicken mAbs reactive for the bovine PrP peptide B204 from one cell fusion experiment.

Based on the comparison of PrP peptides (amino acid residues 204–220) in five mammalian species (ox, mouse, human, sheep and hamster) the amino acid sequences of mouse and human and of sheep and hamster, respectively, are identical and the sequences of four species differed from that of the ox in only two amino acids (Fig. 1). Therefore, production of specific antibodies can not be expected if mouse, sheep or hamster are immunized with the B204 peptide. On the other hand, comparison of PrP peptides (amino acid residues 204–220) between chickens and the above five species, shows that only two amino acids, amino acid residues 205 (Lys) and 220 (Val) are common (Fig. 1).

The results of a competitive ELISA using the 19 mAbs and synthetic peptides showed that these mAbs were divided into five groups. Groups A and B showed broad species cross-reactivity, Group C showed inter-species reactivity with ox, mouse and human, Group D showed inter-species reactivity with ox, sheep and hamster, and Group E reacted specifically with bovine PrP. These five antibody groups included all combinations expected based on comparison of amino acid sequences among the five species examined. Of

Table 1. Summary of the reactivity of 19 chicken mAbs against synthetic bovine PrP

Group	mAb	Reactivity ^{a)} to B204	Competition ^{b)} with					Specificity
			B204	B204'	B211	M211	S211	
A	A4, E11, E12 (3) ^{c)}	+	+	+	–	–	–	ox, mouse, human, sheep, hamster
B	B23, B24 (2)	+	+	–	+	+	+	ox, mouse, human, sheep, hamster
C	E13 (1)	+	+	–	+	+	–	ox, mouse, human
D	B10, B12, C5 (3)	+	+	–	+	–	+	ox, sheep, hamster
E	A2, A3, A6, B5, B9, C3, C4, D21, E18, E20 (10)	+	+	–	+	–	–	ox

a) Determined by ELISA using plate coated with B204-OA. The OD value by ELISA of a total of 19 mAbs to B-204-OA was 0.489 ± 0.194 (mean $\pm$ SD). +: positive reaction. b) Determined by competitive ELISA using plate coated with B204-OA and five synthetic peptides. Briefly, an excess of peptide was added to the supernatant of positive hybridomas, and then incubated for 1 hr at 37°C. The inhibitory activity of the additional peptide to the mAbs examined was determined by an ELISA using B204-OA-coated ELISA plates. In this competitive ELISA, the inhibitory activity of the additional peptide was decided as a positive reaction (+) when the OD value was less than one-hundredth of positive control, while the reaction without thus competitive activity to antibody tested was represented as a negative (–). c) Number of mAbs.

the total of 19 mAbs, however, only three mAbs (Group A) recognized PrP peptide B204' (amino acid residues 204–210) which is completely conserved in the five mammalian species examined. The remaining 16 mAbs (Groups B, C, D and E) recognized PrP peptides B211, M211 and/or S211 (amino acid residues 211–220). Further, 10 of these 16 mAbs were specific for bovine PrP. These results indicate that, in the bovine 17-amino acid peptide (B204, amino acid residues 204–220), the major epitopes against chickens may exist into the later sequence (amino acid residues 211–220). However, as the epitope mapping of 19 mAbs was not carried out, the detailed epitope reactivity of these 19 mAbs requires further clarification.

Mouse mAbs which recognize bovine PrP peptides other than B204 have been already reported, but these mAbs reacted with both bovine and sheep PrP [5]. Therefore, this current report is not only the first of chicken mAbs to bovine PrP but also the first report of mAbs to PrP with bovine species specificity.

In Western blot analysis using 19 mAbs and native bovine PrP^c preparation as a preliminary experiment, no positive reactions were observed for unknown reasons in spite of several trials (unpublished data). Thus, these mAbs may not react with the native PrP^c. Therefore, many of mAbs obtained here may not be useful for further applications for TSE research.

More recently, a mouse mAb 15B3 specific for PrP^{Sc} was generated by using PrP-null mouse and recombinant bovine PrP^c as an immunogen [7]. The result and our results described here indicate that PrP^{Sc}-specific mAb may be generated by using chickens immunized with recombinant PrP without PrP-null animals.

In conclusion, chickens are useful animals for the production of specific antibodies against mammalian PrP peptide antigens and the generation of chicken mAbs appears to be more potentially successful than mouse mAbs for studies of mammalian-conserved antigens such as PrP.

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