MHC-ASSOCIATED RESISTANCE AGAINST MAREK'S DISEASE IN WHITE LEGHORN CHICKENS: REFINED TYPING OF B-G AND B-F ALLELES USING PROTEIN AND DNA ANALYSIS

J.J. Blankert[1], M.G.J. Tilanus[1], B.G. Hepkema[2], G.A.A. Albers[3], E.Egberts[1] and A.J. van der Zijpp[1]

[1]Zodiac Institute, Agricultural University, P.O. Box 338, 6700 AH Wageningen, the Netherlands. [2]Veterinary Faculty, State University, Utrecht, the Netherlands. [3]Euribrid b.v., Boxmeer, the Netherlands

SUMMARY

In a commercial pure White Leghorn line B MHC haplotypes B^{19}, B^{21}, B^{134} and B^{234} were serologically identified. Characteristic patterns of B-G and B-F molecules were obtained in Western blots and IEF gels, using monoclonal antibodies 18-6G2 and F21-2. Southern blots, containing EcoRI restriction fragments and hybridized with B-G and B-F cDNA probes, revealed specific RFLP patterns for all four B haplotypes. In experimental infections with Mareks disease virus, B^{19} was associated with susceptibility, whereas B^{21}, B^{134} and B^{234} were relatively resistant. B^{21} and B^{234}, which were equally resistant, shared identical patterns of B-F molecules in IEF, as well as two B-F restriction fragments. This may indicate that these shared B-F gene(s) were directly involved in disease resistance. Further studies will include more restriction enzymes, as well as additional typing of B-L genes and gene products. Refined typing of B haplotypes may thus enable a more precise localization of resistance gene(s) within the MHC.

INTRODUCTION

The chicken major histocompatibility complex (MHC) is a multigene family encoding class I (B-F), class II (B-L) and class IV (B-G) molecules. A characteristic feature of all chicken MHC (B complex) molecules is extensive polymorphism, originally detected by serological techniques. Remarkable associations between certain B haplotypes and resistance against the virally induced Marek's disease (MD) have been found (Bacon, 1987). In a previous study we have confirmed these results for commercial pure White Leghorn lines, demonstrating B^{21}-associated resistance and B^{19}-associated susceptibility (Blankert et al.. 1990). However, we have not mapped resistance gene(s) within the B complex.
Evidence for B-F-associated, rather than B-G-associated resistance has come from studies using recombinant B haplotypes (Briles et al.. 1983). In order to map MHC-associated disease resistance, more haplotypes should be tested in experimental MD infections, and refined typing of haplotypes should be performed. It has already been demonstrated that, in addition to serotyping, biochemical analysis of B-G and B-F molecules can be performed (Hepkema et al.. 1989), as well as analysis of DNA restriction fragments (Tilanus et al. 1989, Chaussé et al.. 1989). In the present study we have applied these techniques on B serotypes of a commercial pure White Leghorn line B and tested their relative resistance in a challenge experiment.

MATERIAL AND METHODS

Animals. Chickens were from commercial pure White Leghorn line B.
Virus inoculation and assessment of MD. Parental stocks were vaccinated with Marek's disease virus (MDV) strain CVI 988. Birds used for MDV challenge experiments received no vaccines. Spreader chicks, infected by intramuscular injection of cell-associated virulent MDV strain K, were mixed with one-day-old test birds in a 1:20 ratio. Mortality from MD was confirmed by gross examination of

tumors.
Statistics. Experimental data were tested for statistical significance by Student's T-test on transformed proportions (arcsin Vp).
Serological MHC-typing. Direct hemagglutination was performed in round-bottom polystyrene plates. Typing sera were produced by erythrocyte- and lymphocyte-alloimmunizations between line B individuals.
Western blotting. For the isolation of erythrocyte proteins 50 ul of packed cells were incubated in 20 mM Tris-HCl buffer (pH 8), containing 150 mM NaCl, 1 mM $MgCl_2$, 2 % v/v NP-40 and 1 % PMSF during 1 hr on ice. Protein samples were run on 12.5 % SDS-PAGE under reducing conditions. Proteins were blotted to nitrocellulose and B-G molecules were stained with monoclonal antibody 18-6G2 (kindly provided by K. Skjodt and J. Salomonsen, Copenhagen, Denmark), goat anti-mouse IgG (H+L) HRP conjugate and nitroprusside/o-dianisidine substrate.
Immunoprecipitation. Peripheral blood leukocytes were metabolically labelled with ^{35}S-methionin, precipitated with monoclonal antibody F21-2 (K. Skjodt) and separated by IEF as described (Hepkema et al., 1989).
Southern blotting. DNA was isolated from erythrocytes as described (Tilanus et al., 1989). After digestion with EcoRI, DNA fragments were separated on 0.7 % agarose gels. Gels were denatured prior to transfer to Hybond-N nylon membranes. B-G cDNA clone G5 and B-F clone F3 were kindly supplied by J. Kaufman (Basel, Switzerland) and used as probes after radiolabelling with ^{32}P-deoxycytidine triphosphate.

RESULTS

Serologically, four different B haplotypes were detected by antisera raised against allogeneic erythrocytes. Frequencies of the serotypes were 38.4 % for B^{19}, 40.2 % for B^{21}, 5.4 % for B^{134} and 15.4 % for B^{234}. Monospecific antisera for B^{19}, B^{21} and B^{134} were also produced by lymphocyte immunizations. Serotypes B^{19} and B^{21} have been identified as reference B haplotypes by cross-absorptions of antisera (data not shown).
Western blotting analysis of B-G molecules revealed characteristic polymorphism of molecular weights in different alleles. Figure 1A shows that in B^{19} at least three B-G products were detected. In B^{21} only one product was recognized, and in B^{134} three or four. The staining of B-G products in B^{234} was identical with B^{134} (not shown).
Immunoprecipitation and IEF of B-F molecules revealed characteristic patterns for B^{19}, B^{21} and B^{134}; B^{234} could not be discriminated from B^{21} (Figure 1B).
Figure 2 shows that characteristic EcoRI restriction fragment length polymorphism of B-G genes occurred in B^{19}, B^{21}, B^{134} and B^{234}. Polymorphic fragments were shared between maximally two different haplotypes. Polymorphic restriction fragments were also detected when the B-F probe was used, with two fragments shared between B^{21} and B^{234} (Figure 3).
Table 1 shows that in the progeny from B^{19}/B^{21} sires, mated with B^{21}/B^{21} dams, homozygous B^{21} chickens always survived better than B^{19}/B^{21} heterozygotes. Using progeny from B^{19}/B^{134} and B^{19}/B^{234} sires, it was found that mortality in B^{134}/B^{21} or B^{234}/B^{21} heterozygotes was always lower than in B^{19}/B^{21} birds (Table 1). When mortality in B^{134} and B^{234} was compared with B^{21}, no significant difference was found between B^{21}/B^{234} and B^{21}/B^{21} birds; however, B^{21}/B^{134} suffered from a higher mortality rate than B^{21}/B^{21} chickens. In two progenies with segregating B^{134} and B^{234} haplotypes no different mortality rates could be detected for B^{134}/B^{21} and B^{234}/B^{21} birds (Table 1).
In conclusion, it appeared that B^{19} was associated with relatively high mortality from MD, whereas B^{21} and B^{234} were equally resistant; B^{134} was apparently associated with resistance, but differed significantly from B^{21} at least in one family group. Finally, an effect of genetic background was demonstrated because

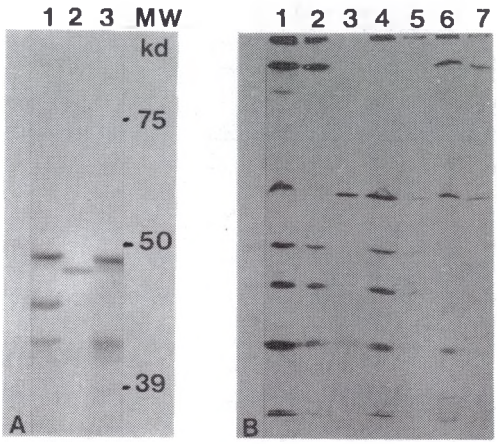

Figure 1. A. B-G molecules from homozygous B^{19} (lane 1), B^{21} (lane 2), B^{134} (lane 3) line B chickens, separated by SDS-PAGE and stained with monoclonal antibody 18-6G2. B. B-F molecules precipitated with monoclonal antibody F21-2 and separated by IEF, obtained from B^{19}/B^{134} (lanes 1 and 2), B^{21}/B^{234} (lane 3), B^{19}/B^{21} (lanes 4 and 5), B^{21}/B^{134} (lanes 6 and 7).

mortality in homozygous B^{21} offspring from one sire (36 %) differed significantly from mortality in B^{21} offspring from other cocks (3, 8 and 9 %; p , 0.05).

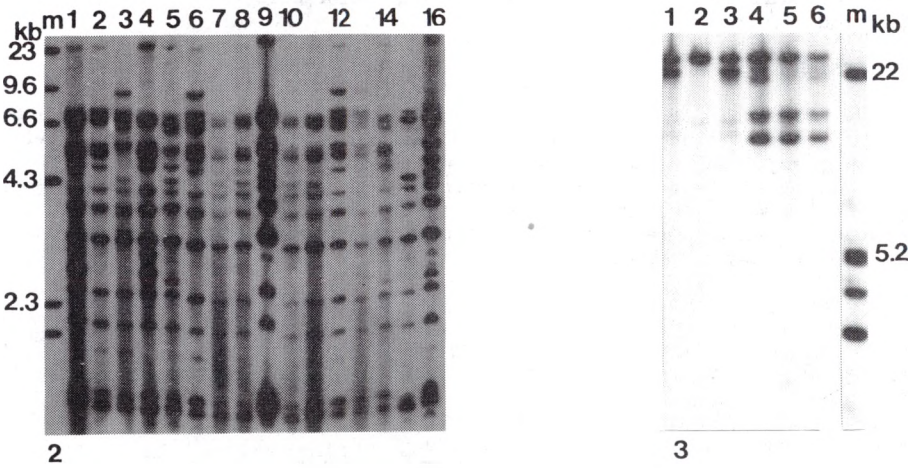

Figure 2. Southern blot of EcoRI-digested genomic erythrocyte DNA, hybridized with B-G cDNA clone G5: B^{21}/B^{234} (lanes 1,2,6,12,13), B^{19}/B^{21} (lane 3), B^{234}/B^{234} (lane 4), B^{19}/B^{134} (lanes 5,16), B^{19}/B^{234} (lanes 7-11), B^{134}/B^{234} (lane 14) and B^{19}/B^{19} (lane 15).

Figure 3. EcoRI-digested erythrocyte DNA, hybridized with B-F cDNA clone F3: B^{19}/B^{134} (lanes 1,3), B^{19}/B^{19} (lane 2), B^{19}/B^{21} (lane 4), B^{19}/B^{234} (lanes 5,6).

Table 1. Mortality of line B chickens after 10 weeks of contact challenge with MDV strain K.

Sire genotype	Dam (n=20) genotype	Progeny genotype	n	Mortality %
B^{19}/B^{21}	B^{21}/B^{21}	B^{19}/B^{21}	31	53[a]
		B^{21}/B^{21}	27	19[b]
B^{19}/B^{21}	B^{21}/B^{21}	B^{19}/B^{21}	21	29[a]
		B^{21}/B^{21}	43	9[b]
B^{19}/B^{134}	B^{21}/B^{21}	B^{19}/B^{21}	45	20[a]
		B^{134}/B^{21}	41	5[b]
B^{19}/B^{234}	B^{21}/B^{21}	B^{19}/B^{21}	55	57[a]
		B^{234}/B^{21}	46	27[b]
B^{21}/B^{134}	B^{21}/B^{21}	B^{21}/B^{21}	38	3[a]
		B^{134}/B^{21}	27	22[b]
B^{21}/B^{234}	B^{21}/B^{21}	B^{21}/B^{21}	42	8[a]
		B^{234}/B^{21}	39	13[a]
B^{21}/B^{234}	B^{21}/B^{21}	B^{21}/B^{21}	48	36[a]
		B^{234}/B^{21}	60	22[a]
B^{134}/B^{234}	B^{21}/B^{21}	B^{134}/B^{21}	17	6[a]
		B^{234}/B^{21}	32	10[a]
B^{134}/B^{234}	B^{21}/B^{21}	B^{134}/B^{21}	43	33[a]
		B^{234}/B^{21}	24	22[a]

[a,b]Within families, percentages with different superscripts differed significantly ($p<0.05$).

DISCUSSION

In the present study we have confirmed earlier data, showing that certain B haplotypes are associated with disease resistance. We have shown that refined B-typing may lead to more accurate mapping of resistance gene(s) within the MHC. Although it appeared that wo restriction fragments, shared between B^{21} and B^{234}, were associateed with resistance, further experiments will have to include more haplotypes, as well as refined typing (using more restriction enzymes) of B-G, B-F and B-L alleles.

REFERENCES

BACON, L. 1987. Poultry Science 66: 802-811.
BRILES, W.E., BRILES, R.W., TAFFS, R.E. and STONE, H.A. 1983. Science 219: 977-979.
CHAUSSE, A.M., COUDERT, F., DAMBRINE, G., GUILLEMOT, F., MILLER, M.M. and AUFFRAY, C. 1989. Immunogenetics 29: 127-130.
HEPKEMA, B.G., VAN DER POEL, A., BLANKERT, H., TILANUS, M.G.J. and HENSEN, E.J. 1989. Rec. Adv. Immunol. Res. 307: 203-211.
LAEMLI,
TILANUS, M.G.J., VRIELINK-VAN GINKEL, R., ENGELEN, I., RIETVELD, F.W., HEPKEMA, B.G., VAN DER ZIJPP, A.J., EGBERTS, E. and BLANKERT, H. 1989. Rec. Adv. Immunol. Res. 307: 187-196.