Immunogenetics 28: 374–379, 1988

Immuno-genetics

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Genotyping chickens for the *B-G* subregion of the major histocompatibility complex using restriction fragment length polymorphisms

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Abstract. Chicken *B-G*-subregion cDNA probes were used to analyze restriction fragment length polymorphisms (RFLP) of the *B-G* subregion of the chicken major histocompatibility complex. Genomic DNA from chickens representing 17 of the 27 standard *B* haplotypes were digested with restriction endonucleases and analyzed in Southern hybridizations with two cDNA clones from the *B-G* subregion. Each *B-G* genotype was found to produce a unique pattern of restriction fragments in these Southern hybridizations. With 15 of the 17 genotypes examined, the different genotypes could be readily distinguished in hybridizations produced with DNA digested with a single restriction enzyme, *PVU* II. The two additional genotypes produced nearly identical patterns in *PVU* II preparations and with three additional enzymes as well, but were readily distinguishable in *Eco* RI digestions. For many of the haplotypes, samples from several individuals in different flocks were examined. In every instance, genotyping by RFLP pattern was found to confirm the *B-G* allele assigned serologically.

Introduction

The *B-G* subregion of the avian major histocompatibility complex (MHC) encodes antigens apparently restricted to the surfaces of erythroid stem cells and erythrocytes (Pink et al. 1977, Longenecker and Mosmann 1980). While clearly linked with the MHC and highly polymorphic, the role of these antigens in immunity, if any, remains to be determined. Indeed, the B-G antigens were first recognized as a blood group (Briles et al. 1950) and then later found to be genetically linked to the transplantation antigens (Schierman and Nordskog 1961) subsequently referred to as B-F (class I) and B-L (class II) antigens by Pink and colleagues (1977). The B-G antigens, now sometimes referred to as class IV antigens, remain of major importance in conventional typing of chickens for MHC haplotypes. This is the result of a combination of factors which include the relative immunogenicity and abundance of the B-G antigens on erythrocytes, the apparent low recombination frequency between *B-G* and the *B-F/B-L* subregions, and the ease of typing by hemagglutination. At the same time, typing by hemagglutination with alloantisera, in particular, often requires knowledge of pedigree and the assiduous attention to the choice of haplotypes used in producing and absorbing the typing sera (see Briles and Briles 1982). Typing with monoclonal antibodies has other difficulties associated with the often highly cross-reactive nature of monoclonal antibodies directed against commonly shared epitopes on polymorphic determinants (Mosmann et al. 1980), although some haplotypes can be typed (Longenecker et al. 1979, Longenecker and Mosmann 1981), and definitive patterns of immunoprecipitated B-G polypeptides can be obtained by high resolution two-dimensional gel electrophoresis (Miller et al. 1984). In this report evidence is presented for an alternate means of genotyping chickens for the *B-G* subregion of the MHC by means of restriction fragment length polymorphisms revealed by two different cDNA probes for genes within the *B-G* subregion.

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Materials and methods

Animals. Blood samples, obtained from multiple sources (see Table 1), were collected into heparinized tubes from birds of serologically defined *B*-system haplotypes. In many instances these were from the same flocks, mostly White Leghorn, used in establishing of a standard *B*-system nomenclature through serological typing (see Briles et al. 1982). An effort was made to obtain representatives of all standard haplotypes. Those listed by Briles and colleagues (1982) and not included in this study had either been lost (B^{27} and B^{29}, W. F. Briles, personal communication) or were not readily available (e. g., the Prague lines).

DNA preparation. High relative mass DNA was isolated from erythrocytes, after first lysing the cells with 10 m*M* Tris-HCl, 10 m*M* KCl, 1.5 m*M* $MgCl_2$, 5 m*M* sodium tetrathionate, pH 8, by digestion with 0.5 mg/ml proteinase K in 50 m*M* Tris-HCl, 100 m*M* sodium ethylenediaminetetraacetate (EDTA), 0.5% sodium dodecyl sulfate overnight at 55 °C (Lacy et al. 1983). The DNA was extracted three times with phenol and twice with chloroform : isoamyl alcohol (24 : 1) and dialyzed against 10 m*M* Tris-HCl, 1 m*M* EDTA, pH 8.

Hybridization probes. Two B-G cDNA clones, bg28, previously described (Goto et al. 1988) and bg32.1, described below, subcloned into Bluescript (Stratagene, San Diego, California) were purified from the vector prior to labeling by random priming (Feinberg and Vogelstein 1983).

Digestion of genomic DNA, electrophoresis, and hybridization. Twenty-microgram samples of genomic DNA were digested with 5 units/μg of restriction enzyme overnight at 37 °C, then precipitated with ethanol. Five-microgram aliquots were subjected to electrophoresis in 0.8% agarose gels. Hybridization was carried out in the gels as described previously (Goto et al. 1988).

Results

bg32.1 cDNA clone. The bg32.1 is a 650-bp cDNA clone isolated from a lambda gt11 expression library made from embryonic erythroid cell mRNA (the M library originally described by Moon et al. 1985). The clone was isolated by cross-hybridization with bg32, a clone originally obtained by screening the same library with antibodies prepared against purified B-G 21 antigen (Goto et al. 1988).

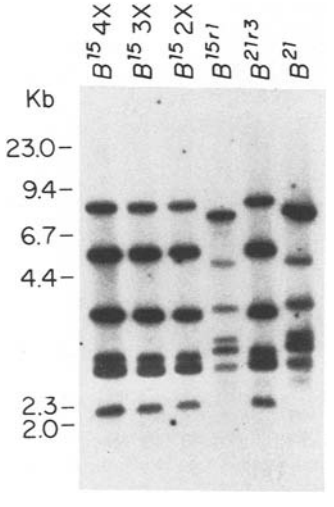

Fig. 1. Southern blot analyses of hybridization between bg32.1 and chicken genomic DNA. DNA samples are from birds of B^{15} haplotype disomic (2×), trisomic (3×), and tetrasomic (4×) for the *B* system-bearing microchromosome and from birds of B^{15r1}, B^{21r3} and B^{21} haplotypes. *Pvu* II-digested genomic DNA samples (5 μg each) were subjected to electrophoresis in a 0.8% agarose gel and hybridized within the gel to ^{32}P-labeled bg32.1 insert. On the *left* are molecular size markers (in kilobase pairs) based on a *Hind* III digestion of phage λ. The autoradiogram is the result of an overnight exposure

Under conditions of high stringency, the bg32 and bg32.1 fragments fail to hybridize with the previously described bg28 clone (Goto et al. 1988). However, as demonstrated previously with bg28, the bg32.1 clone can be assigned to *B* system-bearing microchromosome and further assigned to the *B-G* subregion on the basis of the patterns of hybridization with DNA from birds polysomic for the *B* system-bearing microchromosome and with DNA from MHC recombinant haplotypes (Fig. 1). The intensity of hybridization of the bg32.1 probe to the DNA of polysomic birds increases proportionate to the copy number of the *B* system-bearing microchromosome. The bg32.1 probe can be further assigned to the *B-G* subregion on the basis of the pattern of hybridization with DNA from *B*-system recombinants derived from two independent recombinant events which produced essentially reciprocal rearrangements of the *B-F/B-L* and *B-G* subregions in B^{15} and B^{21} haplotypes. The pattern of hybridization with DNA of the recombinants matches that of the *B-G* subregion contributing parental haplotypes (Fig. 1).

Molecular genotyping for B-G. High relative mass DNA was isolated from blood samples collected from birds of known *B*-system haplotypes carried in several different flocks (see Table 1 and Discussion). Samples were taken from one or more individuals of each flock examined. Illustrated in Figure 2 are the patterns of hybridization between bg28 and bg32.1 and *Pvu* II-digested DNA from a single representative of each of the 17 standard haplotypes examined. Multiple DNA restriction fragments, 4–10 per haplotype, ranging in size from approximately 1–10 kb, are detected by the two probes. Some fragments are common to the patterns produced by both probes. For example, the three largest fragments in the B-G^{21} patterns produced with both probes appear to be identical. Other fragments are detected only by one or the other of the probes. A number of the restriction fragments appear to be widely shared among the haplotypes, although with the exception of perhaps one fragment of about 5.2 kb present in *Pvu* II-digested DNA probe with bg28, none are shared in common across all the haplotypes examined. The *B-G* subregions are each so different, as reflected in the restriction fragment patterns, that generally the different genotypes can be distinguished readily from each other in a Southern hybridization using this single restriction enzyme and either of the two B-G cDNA probes. The only exceptions appear to be the patterns produced by DNA from birds of B^4 and B^{11} haplotypes. The other important finding is that without exception the restriction fragment patterns were the same for each *B-G* allele across the samples included in this study, including samples obtained from different populations known on the basis of serological typing to carry the same *B* haplotypes.

In order to distinguish clearly the *B-G* genotype of B^4 and B^{11} birds, it was necessary to employ additional re-

Table 1. *B-G* genotypes analyzed

B-G allele	*B* haplotype	Line	Status	Figure(s) illustrating	Sample size	Source
$B\text{-}G^2$	B^2	RPRL-15.7-B^2*	C[+]	2	3	East Lansing[≠]
$B\text{-}G^2$	B^2	RPRL-15.6-B^2	I, C	---	3	East Lansing
$B\text{-}G^2$	B^2	UCD-331	I, C	---	3	Davis
$B\text{-}G^2$	B^2	Reference stock	S	---	1	DeKalb
$B\text{-}G^3$	B^3	UCD-313	I, C	2	2	Davis
$B\text{-}G^4$	B^4	PR-CC*	I, C	2, 3	1	Basel
$B\text{-}G^5$	B^5	RPRL-15.15I-B^5*	I	2	2	East Lansing
$B\text{-}G^6$	B^6	G-B2*	I	2	1	Athens
$B\text{-}G^{10}$	B^{10}	Reference stock*	S	2	2	DeKalb
$B\text{-}G^{11}$	B^{11}	Wis 3*	S	2, 3	2	DeKalb
$B\text{-}G^{12}$	B^{12}	PR-CB*	I, C	2	1	Basel
$B\text{-}G^{12}$	B^{12}	RPRL 15.C-B^{12}	I, C	---	2	East Lansing
$B\text{-}G^{13}$	B^{13}	G-B1*	I	2	1	Athens
$B\text{-}G^{13}$	B^{13}	RPRL 15.P-B^{13}	I, C	---	2	East Lansing
$B\text{-}G^{14}$	B^{14}	UCD-316	I, C	2	2	Davis
$B\text{-}G^{15}$	B^{15}	RPRL-15I_5-B^{15}*	I, C	2	2	East Lansing
$B\text{-}G^{15}$	B^{15}	Polysomic	S	1	9	Ithaca
$B\text{-}G^{15}$	B^{15}	UCD-254	I, C	4	2	Davis
$B\text{-}G^{15}$	B^{15}	UCD-011	I	---	2	Davis
$B\text{-}G^{15}$	B^{15}	UCD-057	I	---	2	Davis
$B\text{-}G^{15}$	B^{15}	UCD-035	I	---	1	Davis
$B\text{-}G^{15}$	B^{21r3}	R^5, UCD-386	I, R	---	2	Basel/Davis
$B\text{-}G^{15}$	B^{15}	UCD-396 (B^N)	I	---	1	Davis
$B\text{-}G^{17}$	B^{17}	UCD-003*	I, C	2, 4	4	Davis
$B\text{-}G^{18}$	B^{18}	UCD-253*	I, C	2	2	Davis
$B\text{-}G^{19}$	B^{19}	RPRL.15.P-B^{19}*	I, C	2	2	East Lansing
$B\text{-}G^{19}$	B^{19}	UCD-335	I, C	2	2	Davis
$B\text{-}G^{21}$	B^{21}	RPRL.15N-B^{21}*	I, C	2	3	East Lansing
$B\text{-}G^{21}$	B^{21}	UCD-330	I, C	1	>20	Davis
$B\text{-}G^{21}$	B^{21}	UCD-100 (Australorp)	I	---	5	Davis
$B\text{-}G^{21}$	B^{21}	Ref. stock	S	---	1	DeKalb
$B\text{-}G^{21}$	B^{15rl}	R^4, UCD-387	I, R	1	2	Basel/Davis
$B\text{-}G^{23}$	B^{23}	UNH-105*	S	2	1	DeKalb
$B\text{-}G^{24}$	B^{24}	UNH-105*	S	2	1	DeKalb
$B\text{-}G^{24}$	B^{24}	UCD-312	I	---	1	Davis
$B\text{-}G^C$	B^C	UCD-342 (Ceylonese X Red Jungle Fowl)	I, C	---	1	Davis
$B\text{-}G^J$	B^J	UCD-333 (Red Jungle Fowl)	I	---	1	Davis
$B\text{-}G^O$	B^O	UCD-104	I, C	---	1	Davis
$B\text{-}G^Q$	B^Q	UCD-336 (Red Jungle Fowl)	I	---	1	Davis

* Reference lines used as the type population in standardizing the *B* system nomenclature (see Briles et al. 1982), although RPRL samples are now represented by congenic lines.

[+] C-*B* congenic lines. Three groups of congenic lines were examined. PR-CC and CB are congenic with PR-CB as the base line. RPRL congenic lines are based on RPRL-15I and were produced by 10 or 11 backcrosses onto the 15I line (except for only 7 backcrosses for 15.N-21) and are now maintained in small closed populations. UCD congenic lines were developed with line UCD-003 as the base through five generations of backcrosses, and they too are now maintained in small closed populations. Lines UCD-386 and UCD-387 are new lines into which the B^{15rl} and B^{21r3} (Koch et al. 1983) are being introduced into UCD-003 background. Both are in the first backcross generation. I, inbred, syngeneic; S, segregation for B; R, recombinant *B* haplotype

[≠] Sources: Athens, L. W. Schierman, Department of Avian Medicine, College of Veterinary Medicine, University of Georgia, Athens, Georgia; Basel, J. R. L. Pink, Basel Institute for Immunology, Basel, Switzerland; Ithaca, S. E. Bloom, Department of Poultry and Avian Sciences, Cornell University, Ithaca, New York; Davis, H. Abplanalp, Department of Avian Sciences, University of California, Davis, California; DeKalb, W. E. Briles, Department of Biological Sciences, Northern Illinois University, DeKalb, Illinois; East Lansing, L. D. Bacon, Regional Poultry Research Station, East Lansing, Michigan

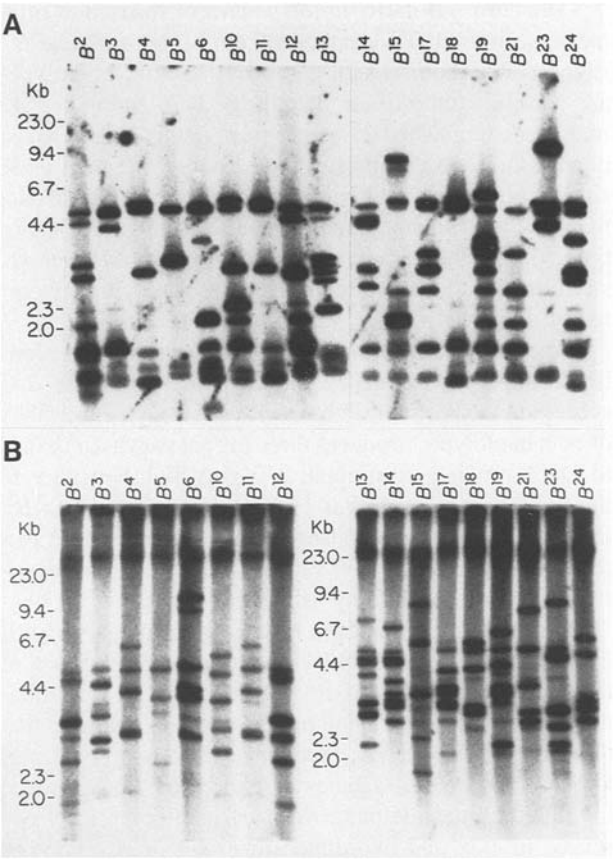

Fig. 2A and B. Hybridization of the bg28 (**A**) and bg32.1 (**B**) probes to restriction digests of chicken genomic DNA from birds of 17 standard haplotypes. *PVU* II-digested genomic DNA (5 μg each sample) were subjected to electrophoresis in a 0.8% agarose gel and hybridized within the gel to the ^{32}P-labeled probes. DNA samples are labeled according to their respective *B* haplotype (see Table 1). Molecular size markers (in kilobase pairs) are based on a *Hind* III digestion of phage λ. The autoradiograms are the results of overnight exposures

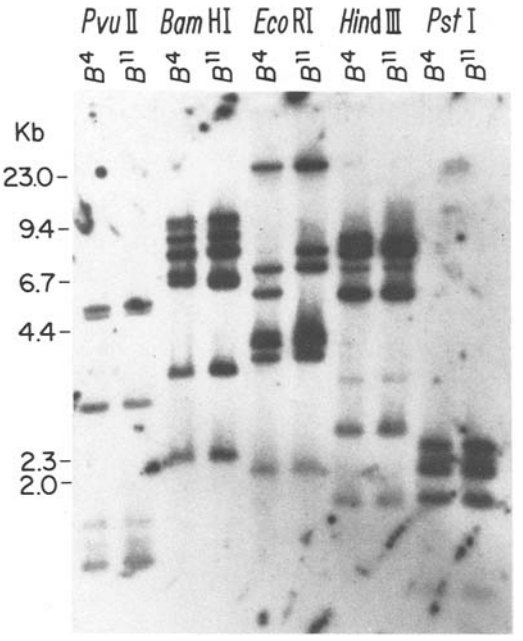

Fig. 3. Hybridization of the bg28 probe to genomic DNA (5 μg each lane) from birds of B^4 and B^{11} haplotypes digested with *Pvu* II, *Bam* HI, *Eco* RI, *Hind* III, and *Pst* I. On the *left* are molecular size markers (in kilobase pairs) based on a *Hind* II digestion of phage λ. The autoradiogram is the result of an overnight exposure

striction enzymes. Among the digestions with five restriction enzymes, only those produced with *Eco* RI provided patterns clearly differentiating these two *B-G* genotypes (Fig. 3). It is notable that even with this enzyme the patterns of the two haplotypes differ only by a proportionate shift in the size of two restriction fragments out of the seven fragments produced.

Given that parental B-G genotype patterns are known or can be determined, it is possible to identify the *B-G* genotypes of heterozygous birds. Illustrated in Figure 4 are the restriction fragment patterns produced with the bg28 probe and *Pvu* II-digested DNA from three generations of birds bearing the *B-G* alleles of B^{15} and B^{17}. The individual restriction fragment patterns of the two parental lines are combined in the pattern of the F_1 generation and can be seen to segregate in individuals representative of the F_2 generation.

B-G-like sequences in previously uncharacterized chicken B system haplotypes and in other avian species. Haplotypes maintained in the flocks at the University of California at Davis originating from the Ceylonese, Grey, and Red Jungle Fowl and assigned temporary alphabetical identifiers produce additional restriction fragment patterns unlike those of the standard serologically defined haplotypes (data not shown). Additional patterns of B-G restriction fragments were found in blood samples drawn from Israeli flocks which are composed primarily of broiler chickens (Miller and Heller, data not shown).

The bg28 probe was also found to hybridize strongly with polymorphic restriction fragments of DNA from several other gallinaceous species including turkey, ring neck pheasant, and golden pheasant. However, the bg28 probe hybridized poorly or not at all with DNA from a number of avian species in other families (data not shown).

Discussion

Finding that each *B* haplotype examined in this study exhibits a unique pattern of *B-G* subregion restriction fragments suggests that Southern hybridizations can provide a means of confirming existing *B-G* subregion genotypes as well as defining new ones. These findings also demon-

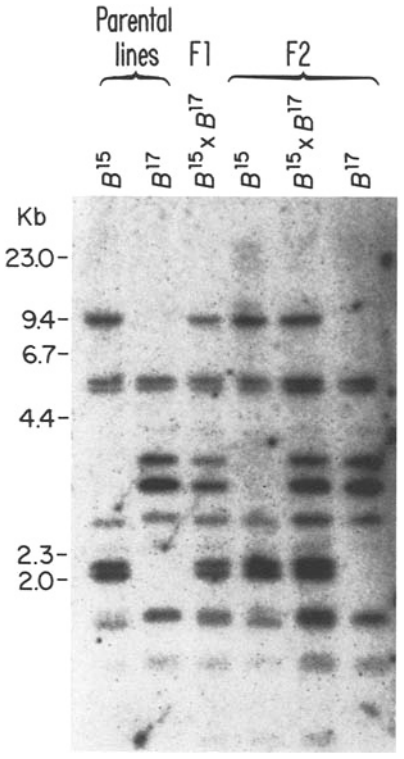

Fig. 4. Hybridization of the bg28 probe to *Pvu* II restriction enzyme digests of genomic DNA (5 μg each sample) from chickens of three generations which includes parents, one representative of the F_1 generation and one representative of each of the possible F_2 genotypes derived from crosses in the F_1 generation. On the left are molecular size markers (in kilobase pairs) based on a *Hind* III digestion of phage λ. The autoradiogram is the result of an overnight exposure

strate the power of the serological methods previously used to define the standard haplotypes. It appears that identity can be extended to the level of restriction fragment length polymorphism between *B-G* subregion loci borne in genetically different lines. In most instances, it is difficult to trace, at length, the lineage of particular inbred lines since many were originally isolated from commercial lines. It seems that most of the different lines carrying standard haplotypes have common origins with perhaps no more than 50 generations since the separation of lines or introduction of particular haplotypes into new flocks. For example, the Reaseheath-Prague CB line-bearing B^{12} haplotype and the RPRL 15.C-12 lines carry the same haplotype obtained from the C line in the 1960s. Similarly, the *B-G*^{15} and *B-G*^{21} alleles each produce the same restriction fragment patterns in all of the B^{15} and B^{21} lines examined, and they may well each have been derived from single genetic sources in the recent past. Descriptions of the origins of inbred and congenic lines trace the history of these and the many other lines (see Abplanalp 1986, Briles and Briles 1982, Hala 1987, and Shen et al. 1984).

The close similarity in the pattern of restriction fragments seen with DNA samples from birds of B^4 and B^{11} suggests that the *B-G* subregions of these two haplotypes are closely similar, even though the two haplotypes are readily distinguished in serological tests (W. E. Briles, unpublished observation). The finding of this close similarity suggests that in instances when serological data may not be available, such as when defining new haplotypes, it may be necessary to probe several different restriction digestions to be confident that genotypes are correctly defined.

Another feature of the B^4 and B^{11} series of digestions is the apparent frequency of *Pst* I sites with the *B-G* subregion DNA. In digestions with this enzyme the DNA of both haplotypes produces three (or possibly four) bands of DNA ranging from about 2–3 kb which hybridize to the bg28 probe. A similar pattern is also seen with B^{21} DNA (M. Miller, unpublished data). This recurrent pattern suggests that the *B-G* subregion may contain repetitive, *Pst* I site-containing sequences.

The patterns of multiple restriction fragments identified by the bg28 and bg32.1 probes are an indication of the genetic complexity of the *B-G* subregion. The number of bands to which bg28 and bg32.1 hybridize suggest that the subregion contains a number of loci. As yet it is not known if multiple *B-G* genes are expressed; however, the multiple polypeptide patterns of varying complexity observed in one- and two-dimensional gel preparations of iodinated, immunoprecipitated B-G antigens suggest that this possibility is likely (Miller et al. 1984, Salomonsen et al. 1987). When a more complete genetic map of the *B-G* subregion is available it should be possible to determine how many genes there may be, whether all genes are expressed, and eventually, which gene sequences might be useful for generating genotype-specific probes that can be used in simplified assays for determining genotype.

Acknowledgments. We thank Larry Bacon, Regional Poultry Research Station, East Lansing; Steve Bloom, Cornell University; Elwood Briles, University of Northern Illinois; Dan Heller, Hebrew University; James Letcher, Santa Barbara Zoological Gardens; Louis Schierman, University of Georgia; and Richard Pink, Hoffman La Roche, Basel, Switzerland for providing us with blood samples. We would also like to thank Garry Miyada for helpful discussions and Jackie Chang for assistance. This research was supported by grant NIH AI21736 from the United States Public Health Service. M.M.M. is a member of the Cancer Center of the City of Hope (NIH CA33572).

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Received June 27, 1988