Haplotypes of the bovine IgG2 heavy gamma chain in tick-resistant and tick-susceptible breeds of cattle

Wanessa Araújo Carvalho · Patricia Ianella · Frederico G. C. Arnoldi · Alexandre Rodrigues Caetano · Sandra Regina Maruyama · Beatriz Rossetti Ferreira · Luís Henrique Andreucci Conti · Marcia Ramos Monteiro da Silva · José Otavio F. Paula · Antonio Augusto Mendes Maia · Isabel K. Ferreira de Miranda Santos

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Abstract Bovines present contrasting, heritable phenotypes of infestations with the cattle tick, *Rhipicephalus (Boophilus) microplus*. Tick salivary glands produce IgG-binding proteins (IGBPs) as a mechanism for escaping from host antibodies that these ectoparasites ingest during blood meals. Allotypes that occur in the constant region of IgG may differ in their capacity to bind with tick IGBPs; this may be reflected by the distribution of distinct allotypes according to phenotypes of tick infestations. In order to test this hypothesis, we investigated the frequency of haplotypes of bovine IgG2 among tick-resistant and tick-susceptible breeds of bovines. Sequencing of the gene coding for the heavy chain of IgG2 from 114 tick-resistant (*Bos taurus indicus*, Nelore breed) and tick-susceptible (*B. t. taurus*, Holstein breed) bovines revealed SNPs that generated 13 different haplotypes, of which 11 were novel and 5 were exclusive of Holstein and 3 of Nelore breeds. Alignment and modeling of coded haplotypes for hinge regions of the bovine IgG2 showed that they differ in the distribution of polar and hydrophobic amino acids and in shape according to the distribution of these amino acids. We also found that there was an association between genotypes of the constant region of the IgG2 heavy chain with phenotypes of tick infestations. These findings open the possibility of investigating if certain IgG allotypes hinder the function of tick IGBPs. If so, they may be markers for breeding for resistance against tick infestations.

Keywords Bovine IgG2 · Allotypes · Haplotypes · Hinge region · *Rhipicephalus (Boophilus) microplus*

Introduction

Allotypic markers of IgG constant heavy chains are encoded by Mendelian co-dominant alleles and have important immunological functions (Grubb 1994). Accordingly, IgG allotypes have been associated with resistance to infections with different microbes (Kacskovics, Wittum, Butler et al. 1995; Corbeil, Gogolewski, Kacskovics et al. 1997; Jönsson, Oxelius, Truedsson et al. 2006; Skattum, Gullstrand, Holmström et al. 2008; Pandey, Namboodiri,
DNA sequences were analyzed sequenced with BigDye terminator chemistry (Applied Biosystems, Carlsbad, CA). DNA sequences were analyzed treated with ExoSAP-IT (Amersham, Piscataway, NJ) and groups show varying levels of resistance (Blankenship, lus (Boophilus)′ PCR with specific primers (5′CGGGGTGTC TGTGAACCA3′ and 5′GTTCCTCAGCCTAATGG3″), treated with ExoSAP-IT (Amershan, Piscataway, NJ) and sequenced with BigDye terminator chemistry (Applied Biosystems, Carlsbad, CA). DNA sequences were analyzed using Phred/Phrap/Consed (Ewing, Hillier, Wendt et al. 1998; Ewing and Green 1998) and PolyPhred (Nickerson, Tobe, and Taylor 1997) tools and nineteen single nucleotide polymorphisms (SNPs) were identified (Table 1). Individual SNP frequencies, estimated by Microsatellite Toolkit (Park 2001), demonstrated that ten SNPs showed breed-specific alleles (P<0.001, Supplemental Table). Haplotypic frequencies of the IgG2 exon fragment, calculated using Arlequin (ELB algorithm; Excoffier, Laval and Schneider 2005), revealed 13 different IgG2 haplotypes, of which 8 were exclusive of taurines, and 2 of indicines (Table 1). RXC software (Miller 1997) demonstrated a highly significant difference in the distribution of haplotypes between taurines and indicines (P=0.000010≤0.000001, Metropolis algorithm). The IgGy2b allotype was significantly more frequent in taurine animals (P<0.0001), while IgGy2b was significantly more frequent in indicine animals (P=0.0001; Table 1).

IgG-binding proteins (IGBs) are a common escape mechanism among pathogens against which antibodies constitute the main effector mechanisms (De Miranda-Santos and Campos-Neto 1981). Certain IgG allotypes are bound less efficiently by IGBPs secreted by Staphylococcus aureus (Van Loghem, Frangione, Recht, and Franklin EC 1982), Haemophilus somnus (Corbeil, Gogolewski, Kacsikovics et al. 1997; Bastida-Corcuera, Nielsen, and Corbeil 1999a), and hepatitis C virus Namboodiri, Budkowska, Nietert, and Pandey 2007 and are associated with a more favorable outcome to infection with the latter pathogens. We show herein that tick IGBPs are abundantly expressed in tick salivary glands. Annotation of a non-normalized cDNA library of R. microplus male salivary glands using BLAST results showed that two contigs contained more transcripts and sequences coding for proteins similar to IGBPs than expected from a random distribution, as evaluated with the χ² test (Table 2). Tick IGBPs may differ in their ability to bind to IgG allotypes compromising the tick’s capacity to modulate host–antibody responses. In order to begin testing these hypotheses, we aligned coded haplotypes of the bovine IgG2 hinge region. Figure 1a shows that haplotypes of the bovine IgG2 hinge region differ in the distribution of polar and hydrophobic amino acids.

We then modeled coded hinge regions of the haplotypes. In silico models were built with Swiss-model repository software (Swiss Institute of Bioinformatics & the Biozentrum, University of Basel, Switzerland) based on an X-ray diffraction of a human IgG1 (1HZH, Protein Data Bank). PyMOL Molecular Graphics System (Schrödinger Corporation, NY, USA) was employed to overlay human IgG1 with the coded haplotypes for bovine IgG2 hinge region obtained with the modeling (Fig. 1b, c). This resulted in five groups of molecules that differed in shape in the hinge region.
according to the presence of polar or hydrophobic amino acids (Figs. 1d–h). Figure 1d shows the template overlaid with the first group (G1) of allo-haplotypes that consists of allotype IgGγ2a (grey), coding residues S, P, N, and H, and a haplotype (green) coding residues S, C, H, and H at positions 219, 224, 225, and 228, respectively. These two haplotypes do not contain hydrophobic amino acids at positions 219 and 228 and are significantly more frequent or found only in taurine animals, respectively (Table 1). Figure 1e shows the template overlaid with the second group of haplotypes, which like those in group G1 do not contain hydrophobic amino acids at positions 219 and 228, but contain a positively charged arginine residue at position 224. It contained one (yellow) coding residues S, R, N, and H and another (pink) coding S, R, H, and H at positions 219, 224, 225, and 228, respectively. These haplotypes were found

Table 1 IgG2 haplotype frequencies in genetically tick-resistant (Nelore, Bos taurus indicus) and tick-susceptible (Holstein, Bos taurus taurus) breeds of cattle

| Gene    | Haplotypea | Amino acid residues in hinge regionb | % Haplotype frequency | P value               |
|---------|------------|-------------------------------------|-----------------------|----------------------|
|         |            |                                     |                       |                      |
|         |            |                                     |                       | Nelore  | Holstein | Fisher exact test   |
| IGHG2   | [G;?:?:?:?]? | –                                   | 0                     | 1.5     | 0.656    |                     |
|         | [T;?:?:?:?]? | –                                   | 2.1                   | 3.0     | 0.674    |                     |
|         | [G:C;C;A;A] | [S;P;N;H]d                          | 14.9                  | 59.8    | <0.0001* |                     |
|         | [G:T;G;C;A] | [S;C;H;H]                            | 0                     | 0.8     | 0.855    |                     |
|         | [G:C;G;A;A] | [S;R;N;H]                            | 0                     | 9.8     | 0.009*   |                     |
|         | [G:C;G;C;A] | [S;R;H;H]                            | 0                     | 0.8     | 0.855    |                     |
|         | [G:C;G;A;C] | [S;R;N;P]                            | 0                     | 3       | 0.250    |                     |
|         | [T;T;G;C;C] | [I;C;H;P]f                           | 78.7                  | 15.1    | <0.001*  |                     |
|         | [G:T;G;C;C] | [S;C;H;P]                            | 0                     | 1.5     | 0.674    |                     |
|         | [T;C;C;A;C] | [I;P;N;P]                            | 0                     | 0.8     | 0.855    |                     |
|         | [T;C;G;A;C] | [I;R;N;P]                            | 0                     | 0.8     | 0.855    |                     |
|         | [T;C;G;C;C] | [I;R;H;P]                            | 2.1                   | 2.3     | 0.686    |                     |
|         | [G:C;G;C;C] | [S;R;H;P]                            | 1.1                   | 0       | 0.862    |                     |
|         | [T;C;C;A;A] | [I;P;N;H]                            | 1.1                   | 0       | 0.862    |                     |
|         | [T;C;G;A;A] | [I;R;N;H]                            | 0                     | 0.8     | 0.855    |                     |

*Indicates that the P-value is significant after a Bonferroni correction, which was performed considering the number of haplotypes for the IGHG2 gene

a Reference haplotype: bovine IgG2 exon B (X16702.1.g: [753G/T; 767C/T; 768C/G; 770A/C])

b Amino acid residues at positions 219, 224, 225 and 228 resulting from SNPs identified at nucleotide positions 753, 767, 768, 770 and 780, respectively

c Sample could not be genotyped at nucleotide positions 768, 770 and 780

d Haplotype corresponds to serologically defined allotype IgGγ2a

e Haplotype corresponds to serologically defined allotype IgGγ2b

gene

Table 2 Distribution of differentially expressed transcripts coding for IgG-binding proteins in a non-normalized cDNA librarya of male salivary glands of Rhipicephalus microplus ticks. The χ2 test was calculated using the average number of ESTs per contig (2.65=3)

| Library | Total number of ESTs | Total number of contigs | Average no. ESTs/contig | E-value | Number of ESTs | Expected | P value χ2 test |
|---------|-----------------------|-------------------------|-------------------------|---------|----------------|----------|----------------|
| MSGRm   | 2,163                 | 817                     | 2.65                    | 9e-084  | 27             | 15       | <0.001         |
| Contig  | Best match to NR protein database |
| # 76    | Immunoglobulin G binding protein C | [Rhipicephalus appendiculatus] gi2352274 | 6e-081  | 25             | 14       | <0.001         |
| # 190   | Immunoglobulin G binding protein B | [Rhipicephalus appendiculatus] gi2352272 |

EST expressed sequence tag. NR non-redundant

a Details of library construction, sequencing, bioinformatic treatment, and contig annotation can be found in Maruyama et al. (2010)

b Male salivary glands of Rhipicephalus microplus
only in taurines at low frequencies (Table 1). Groups 3–5 contained a hydrophobic amino acid at position 219 or 228 or at both positions. The third group, shown in Fig. 1f, contained a single haplotype (pink) coding residues S, R, N, and P at positions 219, 224, 225, and 228, respectively. This haplotype was found only in taurines at low frequencies. The fourth group, shown in Fig. 1g, contained six haplotypes, the allo-haplotype IgG2b (orange), more frequent in indicines and which codes residues I, C, H, and P at positions 219, 224, 225, and 228, respectively. These haplotypes were found in both breeds (Table 1). There are several potential consequences for the structural variations within the hinge regions of IgG2 allotypes. Although the role of the hinge region in complement activation and signaling through Fc receptors is disputed (Brekke, Michaelsen, and Sandlie 1995), it has an essential role in antigen binding (Oi,
haplotypes, Mann bovine IgG2b has more than double complement activity good complement activator depending on the allotype: is not homologous, has a very small hinge region, but is a subclass examined in this work and with which human IgG2 IgG2 is a poor activator of complement, bovine IgG2, the SPNH/SPNH (IgG\(^\gamma\)2a) haplotypes (IgG\(^\gamma\)2b) (Bastida-Corcuera, Butler, Yahiro, and Corbeil 1999b). The role of structural modifications in the hinge region and IgG effector functions has not been examined by the few studies on associations between IgG allotypes and complement activity (Skattum, Gullstrand, Holmström et al. 2008; Jönsson, Oxelius, Truedsson et al. 2006) or Fc receptor dependent-effector mechanisms (Kumpel, Wiener, Urbaniak, and Bradley 1989), although one study did examine hinge flexibility of IgG2 allotypes with molecular plots (Bastida-Corcuera, Butler, Yahiro, and Corbeil 1999b).

We next examined if there was an association between genotypes of the constant region of the IgG2 heavy chain with phenotypes of tick infestations. Randomly selected bovines (Nelore=9; Holstein=8) were genotyped and managed together in a tick-infested pasture for 12 months; the numbers of female ticks larger than 4 mm were counted on one side of each animal 13 times at monthly intervals and the average number of ticks/year for each breed and group of genotypes was obtained. Interestingly, we found that taurine animals containing at least one allo-haplotype for IgG\(^\gamma\)2a presented significantly \((P=0.044)\) more feeding female ticks than the single taurine-host homozygous for the IgG\(^\gamma\)2b allo-haplotype (40.62±35.04 and 24.15±24.01 ticks/animal, respectively; Table 3). While indicine animals always presented significantly \((P<0.001)\) less feeding female ticks than taurine animals (an average of 3.45±5.22 versus 38.56±34.20 ticks/animal, respectively; Table 3), the two heterozygous indicine animals also presented significantly \((P<0.001)\) more feeding ticks than individual homozygous for the IgG\(^\gamma\)2b allo-haplotype (6.73±6.60 and 2.91±4.67 feeding ticks/animal, respectively; Table 3). Due to allelic exclusion, both heavy chains of half of the antibodies produced by animals that are heterozygous for the IgG\(^\gamma\)2b allo-haplotype will bear this marker. Admitting that tick IGBPs bind this allo-haplotype more efficiently, our data suggests that a threshold of 50% IGBP-bound antibodies is still sufficient to allow ticks to complete a blood meal. It is also reasonable to suggest that this haplotype could constitute a marker for susceptibility to tick infestations.

In summary, we have described a PCR-based genotyping method and new haplotypes of the bovine IgG2 heavy chain. We observed differences in the polarity of amino acids of the hinge region of distinct haplotypes that may affect interactions of bovine IgG2 with host neonatal Fc receptors (FcRns) and tick IGBPs. Our results indicate that certain bovine allo-haplotypes are associated with phenotypes of infestations with \(R.\ microplus\). Interestingly, we have shown that after heavy exposure to \(R.\ microplus\), the levels of anti-tick saliva IgG2 antibodies remain the same in indicine cattle and decrease in taurines even though these latter animals receive larger quantities of potentially antigenic tick saliva (Kashino, Resende, Sacco et al. 2005). This phenomenon may result from differing biological properties of IgG2 allotypes and merits further investigation. Levels of IgG2 are highly heritable in cattle (Mazengera, Kennedy, Burnside et al. 1985) and are also heritable in humans according to allotypes (Oxelius 1993). This may explain in part our previous findings. Additionally, FcRn which protect endocytosed IgG from degradation and prolong its half-life in the circulation of cattle (Cervenak and Kacskovics 2009) differ in their capacity to bind to allotypes of IgG (West and Bjorkman 2000). Indeed, the levels and catabolic rates of IgG2 differ according to their allotypes (Oxelius and Eibl 1996). FcRns may also differ in their capacity to bind to IgG coated with an IGBP. Taken together, our findings suggest that antibodies are crucial to control tick infestations and that allotypic variation may have important consequences for the host.

**Table 3** Association of allo-haplotypes of the constant region of IgG2 heavy chain with phenotypes of tick infestations

| Allo-haplotype | No. allo-haplotypes/breed | No.±SD of female ticks |
|----------------|---------------------------|-------------------------|
|                | Nelore | Holstein | Nelore | Holstein |
| SPNH/SPNH (IgG\(^\gamma\)2a homozygous) | 0 | 3 | – | 43.46±39.34 |
| SPNH/G4 haplotypes (IgG\(^\gamma\)2a heterozygous) | 2 | 4 | 6.73±6.60 | 38.48±31.65 |
| Total allo-haplotypes containing IgG\(^\gamma\)2a | 2 | 7 | 6.73±6.60 | 40.62±35.04 |
| ICHP/ICHP (IgG\(^\gamma\)2b homozygous) | 7 | 1 | 2.91±4.67 | 24.15±24.01 |
| \(P\) value | 0.015\(^b\) | <0.001\(^b\) | 0.044\(^b\) |

\(^a\)Comparison of the proportion of allo-haplotypes containing IgG\(^\gamma\)2a in two bovine breeds, Fisher Exact Test

\(^b\)Comparison of the median numbers of ticks between animals bearing allo-haplotypes containing IgG\(^\gamma\)2a and animals homozygous for IgG\(^\gamma\)2b allo-haplotypes, Mann–Whitney Rank Sum Test
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