Abstract To determine whether canine rheumatoid arthritis (CRA) is associated with dog MHC (DLA-DRB1) alleles which contain the QRRAA/RKRAA conserved third hypervariable region (3HVR) sequence, DNA samples were extracted from 61 dogs with clinically diagnosed small-joint polyarthritis and from 425 controls. Breed-matched controls were available for 41 cases. DLA-DRB1 genotypes were identified using molecular typing methods. Phenotype frequencies were compared between cases and controls and odds ratios with 95% confidence intervals calculated. Several DLA-DRB1 alleles were associated with increased risk for CRA: DLA-DRB1*002, DRB1*009, and DRB1*018. This was also observed for the presence of any shared epitope (SE)-bearing allele. The associations with DLA-DRB1*002 and the SE were maintained when only breed-matched cases and controls were compared. This study suggests that a conserved amino acid motif in the 3HVR present in some DRB1 alleles of both dogs and humans is associated with rheumatoid arthritis in both species.

Keywords DLA-DRB1 · Dog · MHC · Rheumatoid arthritis · Shared epitope

Introduction

In humans, the etiology of rheumatoid arthritis (RA) is known to have both a genetic and an environmental component. The genetic basis of RA is estimated to account for over 50% of RA susceptibility and to be oligogenic in nature. Approximately one-third of this genetic component can be explained by HLA (Ollier and Worthington 1997). A current view is that RA susceptibility is associated with the presence of a conserved sequence of amino acids (QKRAA/QRRAA/RRRAA) present in the third hypervariable region (3HVR) of a number of HLA-DRB1 alleles (DRB1*0401, *0404, *0405, *0408, *0101, *0102, *1402, and *1001). This sequence is usually referred to as being the RA shared epitope (SE), and a working hypothesis suggests it explains RA susceptibility in different population groups (Gregerson et al. 1987).

Various animal models have been proposed as possible paradigms for RA (Goldings and Jasin 1993). These include type II collagen-induced arthritis in mice and rats, and adjuvant- and oil-induced arthritis in rodents. Most of these induced models only display some features of human RA and although the pathological processes shared between species may have some common genetic basis, such models are unlikely to completely mirror human RA.

Spontaneously occurring animal disease models of autoimmunity will possibly have a greater genetic similarity to their human counterparts. Spontaneously occurring inflammatory polyarthritis has not been clearly described in many animal species. One exception is the dog, in which canine rheumatoid arthritis (CRA) has been well defined by clinical and laboratory criteria (Bennett 1987). This condition in dogs exhibits considerable clinical heterogeneity but is often symmetrical, affecting small joints of the front and hind limbs. In a proportion of dogs, the arthritis is accompanied by pannus formation in the joint and lymphocytic infiltration. In some dogs, the clinical picture is similar to that in human erosive and rheumatoid factor (RF)-positive RA. The autoimmune basis of this disorder is well characterized, with reports of RF (Carter et al. 1989), anti-collagen antibodies (Bari et al. 1989), lymphocytic infiltration of tissues (May et al. 1994), and increased MHC expression...
in synovial tissues (Hewicker-Trautwin et al. 1999). This condition is usually treated with steroids or other antiinflammatory agents. Anecdotally, CRA does not appear to be particularly restricted to a given breed, although no epidemiology-based studies have been conducted to confirm this.

Recently, DLA-DRB1, the dog homologue of the human HLA-DRB1 gene, has been characterized, and fifty-two DLA-DRB1 alleles have been defined so far (Kennedy et al., in press). Great variation in the distribution of DLA-DRB1 phenotype frequencies exists between different dog breeds (Kennedy et al. 1999b). Dog and human DRB1 sequences show an average 86% nucleotide homology, with three HVR defined at the same codons in both species. There is no crystal structure available for DLA-DRB1, but sequence homology suggests that it will be highly similar to the HLA-DRB1 molecule. Examination of DLA-DRB1 sequence alignments has revealed that several alleles, DLA-DRB1*002, *009, *011, and *02501, contain a 3HVR sequence (QRRAA) identical to that in the RA-associated alleles HLA-DRB1*0404, *0405, *0408, *0101, and *1402. In addition, other DLA-DRB1 alleles, *00301, *00801, *00802, *00901, and *02901, contain the 3HVR sequence RRRAA which although not found in human HLA-DRB1 alleles is highly conserved with the RA SE with respect to charge and tertiary structure. Figure 1 shows the amino acid sequence of HVR3 for the DLA-DRB1 alleles identified in this study. Those carrying the shared epitope are indicated in bold. Some other rare DLA-DRB1 alleles carry the RA SE sequences QKRAA and RRRAA, but no dogs in this study had these alleles. We therefore tested the hypothesis that spontaneously occurring CRA is associated with the same conserved class II MHC sequence as in human RA.

Material and methods

Sixty-three dogs with a confirmed clinical diagnosis of small-joint polyarthritis were recruited as referrals to either Liverpool University Small Animal Hospital, The University of Glasgow Veterinary School, or IDEXX Diagnostic Pathology Laboratories UK. Breed was recorded and wherever possible, age at disease onset, radiological assessment, and RF status. Blood samples were also available for 425 control dogs referred for routine hematological investigations. All investigations were performed on residual pathology diagnostic samples and as such did not require a government animal research licence. Table 1 summarizes the data collected for the patient group.

DNA was extracted from residual EDTA anticoagulated blood samples taken as part of routine clinical pathology investigations. Extraction was achieved using a magnetic bead-based method (Dynal, UK). DLA-DRB1 alleles were identified using a PCR-based typing system utilizing nonradioactive sequence-specific oligonucleotide probing (SSOP) (Kennedy et al. 1999a). For animals in which DLA-DRB1 alleles could not be confidently assigned by SSOP, a DNA sequence-based typing approach was followed. Phenotype frequencies were calculated and compared between all cases and controls using $\chi^2$-analysis or Fisher’s exact test. Odds ratios were calculated where appropriate together with 95% confidence intervals. Due to the extremely restricted distribution of DLA-DRB1 allele frequencies observed in some breeds, a more rigorous analysis was also performed; here only CRA cases ($n=41$) from 24 different breeds were included where a breed-matched control could also be identified. For most CRA cases, two breed-matched controls were included, although for a few cases only one such control could be identified ($n=78$).

Results

When CRA cases were compared with all controls (Table 2), two of the SE-positive DLA-DRB1 alleles were associated with CRA, DLA-DRB1*002 ($P=0.02$) and DLA-DRB1*009 ($P=0.001$). DLA-DRB1*018 was also at significantly increased frequency in all CRA cases ($P=0.003$) although this allele does not carry the RA SE. When the frequency of all SE-bearing alleles (DLA-DRB1*002, *008, *009, *011) was examined, a higher frequency of the SE was observed in CRA cases (36.5 vs 21.9%, $P=0.01$). Further analysis, restricted to breed-matched CRA cases and controls, showed significant disease associations only for DLA-DRB1*002 ($P=0.03$) and all SE-bearing alleles ($P=0.04$).

### Table 1 Summary of data collected for the patient group

<table>
<thead>
<tr>
<th>Canine rheumatoid arthritis</th>
<th>Number of cases</th>
<th>Number of different breeds</th>
<th>Female:male ratio</th>
<th>Rheumatoid factor positive</th>
<th>Erosive disease</th>
<th>Number of cases with breed matched controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>63</td>
<td></td>
<td>1.4:1</td>
<td>43%</td>
<td>22%</td>
<td>41</td>
</tr>
</tbody>
</table>

Fig. 1 HVR3 amino acid sequences for DLA-DRB1 alleles identified in this study.