Association of HLA-DRB1 alleles with giant cell tumour of bone

S S Varanasi, N A Athanasou, I Briceno, S S Papiha, H K Datta

Abstract

**Aim**—To examine the possible influence of the MHC class II antigens alleles in the formation of the multinucleate aggressive giant cell tumour of bone.

**Methods**—HLA class II antigen alleles were investigated in eight white patients from north east England with confirmed diagnosis of giant cell tumour of bone. All had locally aggressive, immunophenotypically HLA-DR negative giant cell tumours.

**Results**—Five of the eight patients were found to be positive for HLA-DRB1*0801/3, the distribution of this allele in healthy white controls being quite low (2%). All but one of the patients possessing DRB1*080 also expressed DRB1*070.

**Conclusions**—HLA-DRB1*080 is predominant in patients with immunophenotypic HLA-DR negative giant cell tumours; individuals with the genotype 070/080 are at particularly high risk of developing giant cell tumour of bone.

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Keywords: HLA-DR genotyping; giant cell tumour of bone; osteoclastogenesis

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Giant cell tumour of bone, constituting 5% of all bone tumours, commonly occurs in the third and fourth decade of life. It is a potentially aggressive stromal growth capable of causing considerable damage owing to its bone resorbing capacity. Although occasionally known to metastasise, most tend to produce damage at the primary site. The aetiology of these tumours is unknown and there is no evidence of familial occurrence.

In recent years characterisation studies have attempted to classify giant cells into two main subtypes on the basis of their resorptive activity, calcitonin responsiveness, and HLA-DR immunophenotypic status. It has been suggested that giant cells showing avid bone resorptive activity tend to be HLA-DR negative and are sensitive to inhibition by calcitonin, while cells that are HLA-DR positive lack resorptive activity and are insensitive to calcitonin. However, exceptions to this association have been reported, with immunophenotypic HLA-DR positivity and responsiveness to calcitonin. This apparent association between the HLA-DR and giant cell tumour of bone led us to examine the possible influence of the MHC class II antigen alleles in the formation of aggressive multinucleate giant cell tumours.

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**Methods**

**SELECTION OF PATIENTS**

We selected eight white British patients from north east England with a confirmed diagnosis of locally destructive giant cell tumour of bone. The samples consisted of biopsies collected for pathological examination from patients undergoing surgery for resection of the tumour. The tumours selected did not express HLA-DR antigen and showed evidence of extensive bone resorption.

**HLA-DR GENOTYPING**

Embedded tissue was scraped free from the paraffin blocks and washed in xylol. The remnant bone and soft tissue were suspended in two volumes of ethanol, agitated, and collected by centrifugation. Liquid nitrogen was added to the tissue and the supercooled bone was crushed into smaller pieces. The crushed bone was incubated at 37°C for three days in ESP solution (0.5 mM ethylene diamine tetra-acetic acid (pH 9), 0.019 mg/ml sodium dodecyl sulphate (SDS), 1 mg/ml proteinase K). Genomic DNA was extracted by...
**Table 1** The sequence of sense and antisense of DRB1 oligonucleotide probes

<table>
<thead>
<tr>
<th>DRB1</th>
<th>Sequence</th>
<th>Amino acid position</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 0102, 1201, DRB5*0201, 0202</td>
<td>CTACGGGGCTGTGGAGA</td>
<td>82 Sense</td>
</tr>
<tr>
<td>2. 0401-09</td>
<td>ACCTCTATCTACACCAAGG</td>
<td>30 Sense</td>
</tr>
<tr>
<td>3. 0405, 0409, 0801 (0803, 1303)</td>
<td>GCCTGCTGCGGCGCT</td>
<td>54 Sense</td>
</tr>
<tr>
<td>4. 1201</td>
<td>AGGGGAGCTTCATGGGCA</td>
<td>34 Sense</td>
</tr>
<tr>
<td>5. 0701, 0702</td>
<td>AACGGCTAGGGCGGCCC</td>
<td>51 Sense</td>
</tr>
<tr>
<td>6. 0901</td>
<td>TGCCGGTTCTTGCAGAGA</td>
<td>24 Sense</td>
</tr>
<tr>
<td>7. 0801, 0802, 0803</td>
<td>GCCTGGGCTGCTGGAGA</td>
<td>71 Sense</td>
</tr>
<tr>
<td>8. 1001</td>
<td>GAGCGGAGGCTGGGC</td>
<td>69 Sense</td>
</tr>
<tr>
<td>9. DRB1 1401</td>
<td>CTGCGGAGGCTGGGAC</td>
<td>57 Sense</td>
</tr>
<tr>
<td>10. 0101, 0102, 0103</td>
<td>ATATGACCATCTTTCCA</td>
<td>32 Antisense</td>
</tr>
<tr>
<td>11. 0102, 1201, 1401</td>
<td>TGTCCGTCAATAGGTTGC</td>
<td>81 Antisense</td>
</tr>
<tr>
<td>12. 1501, 1502, DRB5*0201, 0202</td>
<td>CGGCGGCGCGCTGTCGCC</td>
<td>74 Antisense</td>
</tr>
<tr>
<td>13. 0301, 0302</td>
<td>TGCCCTGCTGCA</td>
<td>80 Antisense</td>
</tr>
<tr>
<td>14. 0401, 0409</td>
<td>CACCGGGCGGCGCTGTCGCC</td>
<td>75 Antisense</td>
</tr>
<tr>
<td>15. 0103, 0104, 1301-12, 1102-3</td>
<td>CCCGTCTGCTCAGGG</td>
<td>73 Antisense</td>
</tr>
<tr>
<td>16. 0101, 0102, 0403-08, 1402</td>
<td>CGGCCGCGCTGTCGCC</td>
<td>74 Antisense</td>
</tr>
<tr>
<td>17. 1101, 1102, 1103, 1104</td>
<td>AGTCTTCATGAGC</td>
<td>41 Antisense</td>
</tr>
<tr>
<td>18. 0302, 1402, DRB5*0301</td>
<td>GAAGATCTCTCCAGGA</td>
<td>31 Antisense</td>
</tr>
<tr>
<td>19. 0301, 0302, 1301, 1302, 1402</td>
<td>CGGCGGCGCTCCT GCCGCC</td>
<td>39 Antisense</td>
</tr>
</tbody>
</table>

Adding 1:1 (vol/vol) phenol/chloroform to one volume of ESP extract. The mixture was centrifuged and DNA precipitated from the aqueous phase. Further purification of the genomic DNA was carried out using the Wizard DNA Clean-up System (Promega).

Genotyping was done using polymerase chain reaction (PCR), followed by sequence specific oligonucleotide (SSO) analysis (fig 1). The PCR amplification of the genomic DNA was carried out using the following forward and reverse primers: 5'-TGTCATTTTCTA CTTGAGGC and 5'-TCGGCCGCCTGTC, respectively. The thermocycling parameters used for the amplification of genomic DNA were: denaturation for 30 seconds at 93°C, annealing for one minute at 55°C, and extension for two minutes at 72°C.

Approximately 0.5 µg of the PCR amplification product was subjected to electrophoresis in agarose gel (2%) and treated with denaturating solution followed by neutralising solution for one hour at room temperature. The blotting was performed overnight, DNA transferred to nylon filters (Hybond N) and probed with 32P labelled oligonucleotide probes (table 1). The probes were labelled using [³²P] ATP (60 µCi), 10 pmol of oligonucleotide, and T4 polynucleotide kinase (NBL).

Following hybridisation, the membranes were washed twice at room temperature in 2x SSC (sodium chloride/sodium citrate) / 0.1% SDS, and then in 100 ml of tetramethyl ammonium chloride (TMAC) solution for 10 minutes; thereafter a final wash in TMAC was carried out at 56°C. For reprobing, the filters were strip washed by immersing the filter in 0.4 M NaOH at 42°C for 20 minutes followed by agitation in 0.2 M HCl (pH 8.0), 0.1 × SSPE (NaCl/NaH₂PO₄/EDTA) / 0.1% SDS for 20 minutes. The detection procedure was that recommended by The International Histocompatibility Workshop (Kimura and Suzuki, 1992). The oligonucleotide primers for PCR, SSO analysis were purchased from BSHI (the British Society for Histocompatibility and Immunogenetics, Bristol, UK).

**Results**

Two main DRB1 alleles observed in the patients were *080 and *070. Five of eight patients with giant cell tumours of bone were found to be positive for DRB1*0801/3 allele and four patients were positive for DRB1*070. In the patients, the gene frequency of *0801/3 was 0.380, which is 20-fold higher than the reported genotype frequency in British whites. Other DRB1 alleles observed in the patients were *120, *140, and *010. The frequency of DRB1 alleles in the patients is compared with that in the normal population in table 2.

**Discussion**

Although our study was performed on a comparatively small sample size, it shows a strong association with HLA-DRB1*080 alleles. All but one patient with HLA-DRB1*080 was found to have *070, suggesting that the 070/080 genotype may be associated with a particularly strong predisposition to HLA-DR negative giant cell tumour of bone. The other genotype observed in two patients was 120/140. The frequencies of *120 and *140 in the control population are known to be 0.014 and 0.028, respectively, while in the patients studied the frequency of these alleles was found to be 0.125 (table 2).

These data support a clear association between HLA-DRB1*080 and the formation of giant cell tumours of bone. The predominant haplotypes observed in our patients are those that have been identified in autoimmune disease but were not present in healthy controls. This observation raises a number of issues relating to the classification as well as the aetiology of giant cell tumour of bone. For instance, whether possessing the *080 allele increases the risk of giant cell tumour...
formation in bone, or of multinucleate giant cell tumours in other tissues, needs to be established in a larger study. Further studies are needed to determine whether the observed association between giant cell tumour of bone and DRB1 is likely to occur in other giant cell lesions of bone and soft tissues.

The demonstration of a predominance of a particular HLA type II antigen in individuals at risk of developing giant cell tumour of bone is of interest in view of the recent developments in the area of osteoclast-like multinucleate cell formation. These recent studies have shown that the cytokines TRANCE/RANKL (tumour necrosis factor (TNF) related, activation induced cytokine), which is produced by osteoblasts and T lymphocytes, and M-CSF, which is produced by macrophages and other inflammatory cells, are essential for osteoclastogenesis. The mononuclear stromal cell component of giant cell tumour of bone is shown to contain largely osteoblast-like cells and macrophages. Therefore surface molecules, such as HLA class II antigen, may be crucial in affecting the release of cytokines and the formation of osteoclasts and osteoclast-like giant cells in giant cell tumour of bone.

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