

HETEROZYGOSITY AT Gm LOCI ASSOCIATED WITH  
HUMORAL IMMUNITY TO OSTEOSARCOMA\*

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Familial clustering of osteosarcoma suggests the involvement of genetic factors (1, 2), and the demonstration of a high incidence of osteosarcoma-specific antibodies (3, 4), as well as tumor-specific cell-mediated immunity (5) in patients and their relatives, indicates the involvement of immunological factors in the pathogenesis of this disease. Certain Gm allotypes (genetic markers of IgG) have been shown to be associated with a high relative risk of some forms of cancer. For instance, in Caucasians an unusual Gm haplotype—Gm 1,3;5,13,14—has been found to be associated with neuroblastoma (6), and an increased frequency of Gm (2) has been reported in patients with malignant melanoma (7, 8). A recent report has shown an association of the Gm 1,2;13,15,16,21 phenotype with lung cancer and primary hepatoma in the Japanese (9). To our knowledge, however, the possible role of Gm allotypes in predisposition to osteosarcoma has not been examined.

Immune responsiveness to a variety of antigens in both experimental animals and humans has been shown to be controlled either by major histocompatibility complex (MHC)-linked immune response (Ir) genes or by allotype-linked Ir genes (10–13). In some instances an interactive effect of these two unlinked genetic systems has been observed (12). It is possible that MHC-linked or allotype-linked Ir genes may also influence humoral immunity to tumor antigens. In this report we present evidence for complementary Ir genes controlling immune responses to osteosarcoma-associated antigens (OSAA).

### Materials and Methods

*Patients.* Blood was collected from 50 Caucasian patients with osteosarcoma at the Medical University of South Carolina, Charleston, SC, and the Veterans Administration Medical Center, Gainesville, FL. Diagnosis of osteosarcoma was confirmed after examination of biopsy specimens. All patients had osteoblastic sarcoma. They did not include fibroblastic or chondroblastic variants.

*Cell Lines.* Osteosarcoma cell lines TE-85 and LM were used. These are biologically proven osteosarcoma cell lines by criteria of immunofluorescence, fine structure histology, karyotype analysis, and enzyme activities (14, 15). They do not react with anti-A, anti-B, or anti-AB human blood group antisera, or with antisera for HLA-A, B, C, and DR antigens. Several

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TABLE I  
*Distribution of Gm Phenotypes in Osteosarcoma Patients (n = 50) and  
 Normal Controls (n = 906)*

Phenotype	Incidence	
	Osteosarcoma patients	Normal controls
	%	
Gm 1,17;21	6	3
Gm 1,2,17;21	6	4
Gm 3;5,13,14	36	47
Gm 1,3,17;5,13,14,21	44	31
Gm 1,2,3,17;5,13,14,21	8	14

TABLE II  
*Distribution of Gm Phenotypes and Antibody Response to OSAA in  
 Osteosarcoma Patients*

Phenotype	OSAA-specific antibody	
	+	-
	(%)	
Gm 1,17;21	0	3 (11)
Gm 1,2,17;21	1 (4)	2 (7)
Gm 3;5,13,14	1 (8)	17 (63)*
Gm 1,3,17;5,13,14,21	19 (83)	3 (11)*
Gm 1,2,3,17;5,13,14,21	2 (4)	2 (7)

\*  $P < 0.00001$ .

other cell lines were used as controls: WI-38 (embryonic lung), TE-32 (rhabdomyosarcoma), CAMA-1 and SW-527 (breast carcinoma), and M-14 (melanoma).

*Detection of Antibodies to OSAA.* Sera (diluted 1:20) from 50 patients with osteosarcoma were assayed for antibodies directed against OSAA by the indirect immunofluorescence test described previously (16). Sera showing strong membrane immunofluorescence with TE-85 and LM cells were considered positive and those showing no immunofluorescence were considered negative for antibodies to OSAA.

*Gm Allotyping.* Serum samples were typed for Gm antigens 1(a), 2(x), 3(f), 5(bl), 6(c3), 13(b3), 14(b4), 17(z), and 21(g) by our standard hemagglutination inhibition method (17). Allotyping and detection of antibodies were done in a double-blind fashion.

*Statistical Analysis.* Data were analyzed by  $2 \times 2$  and  $2 \times 5$  contingency chi-square tests.

## Results

The frequencies of various Gm phenotypes in osteosarcoma patients, compared with those previously published for the normal population (8) are shown in Table I. Five Gm phenotypes, all commonly found in Caucasians, were present. None of the phenotypes was significantly associated with osteosarcoma. The distribution of various Gm phenotypes with respect to the presence or absence of antibodies to OSAA is shown in Table II. Two Gm phenotypes—Gm 3;5,13,14 and Gm 1,3,17;5,13,14,21—were significantly associated with immune response to OSAA; however, the associations for the two phenotypes were in opposite directions. Gm 3;5,13,14 was associated with no detectable antibody (8% positive and 63% negative), whereas Gm 1,3,17;5,13,14,21 was clearly associated with detectable antibodies to OSAA (83% positive and 11% negative). Both were highly significant even after correction for the number of comparisons made.

### Discussion

The finding that the distribution of the Gm phenotypes detected in our patient population was similar to that in the normal population suggests that Gm allotypes are not involved in the pathogenesis of osteosarcoma, and that if disease susceptibility genes for osteosarcoma exist, they probably are not in linkage disequilibrium with the alleles coding for Gm allotypes. This does not exclude loose or moderate linkage between Gm loci and loci controlling the pathogenesis of osteosarcoma, which can be determined only after linkage analysis in multiple-case families (18, 19).

Of the 50 patients tested for the presence of antibodies to OSAA, 23 were positive. In an earlier report (20), using appropriate control cell lines, we showed that these antibodies are specific for OSAA and are of the IgG class; the anti-OSAA activity of the sera from osteosarcoma patients was abolished after absorption with cultured human osteosarcoma cells from line LM, TE-85, or G292, but not after absorption with cells from line WI-38 (embryonic lung), TE-32 (rhabdomyosarcoma), CAMA-1 and SW-527 (breast carcinoma), or M-14 (melanoma). These antibodies apparently are not directed toward fetal-type antigens because no membrane immunofluorescence was seen with M-14 cells, which are known to express fetal-type antigens (21). In addition, TE-85 and LM cells apparently do not express fetal-type antigens (K. Y. Tsang, unpublished observation). In the present study we found significant associations between Gm 3;5,13,14 and unresponsiveness to OSAA and between Gm 1,3,17;5,13,14,21 and responsiveness to OSAA. Because the most probable genotype of the Gm 3;5,13,14 phenotype is Gm 3;5,13,14/Gm 3;5,13,14 and that of Gm 1,3,17;5,13,14,21 is Gm 1,17;21/Gm 3;5,13,14, our results can be explained by postulating two Ir genes in linkage disequilibrium with the Gm 1,17;21 and Gm 3;5,13,14 haplotypes, respectively. Possession of either gene alone, i.e., in Gm 1,17;21/Gm 1,17;21 and Gm 3;5,13,14/Gm 3;5,13,14 homozygotes, would not confer humoral responsiveness to OSAA. However, in the heterozygous condition, i.e., Gm 1,17;21/Gm 3;5,13,14, the genes would complement to permit a humoral response to OSAA. Existence of complementary Ir genes for several antigens has been documented in experimental animals (10).

Two other explanations can also be forwarded to explain these results. First, immune responsiveness to OSAA might be controlled by a single dominant Ir gene, Ir(+), in linkage disequilibrium with the Gm 1,17;21 haplotype and its recessive allele, Ir(-), in linkage disequilibrium with the Gm 3;5,13,14 haplotype. This would explain the association of Gm 3;5,13,14/Gm 3;5,13,14 [Ir(-)/Ir(-)] with unresponsiveness to OSAA and the association of Gm 1,17;21/Gm 3;5,13,14 [Ir(+)/Ir(-)] with responsiveness to OSAA. Alternatively, in addition to Ir genes, immune suppression (Is) genes might be involved. An Is gene in linkage disequilibrium with the Gm 3;5,13,14 haplotype would explain the association of unresponsiveness with Gm 3;5,13,14/Gm 3;5,13,14 [Is(+)/Is(+)] and responsiveness with Gm 1,17;21/Gm 3;5,13,14 [Ir(+)/Is(+)]. (Studies in mice have shown that although both Ir and Is genes are dominant in nature, in Ir/Is heterozygotes responder phenotypes are generally dominant over suppressor phenotypes.) If either of the above explanations were accepted, then it would follow that Gm 1,17;21 should be associated with responsiveness to OSAA. Although none of the three patients in this study who were positive for Gm 1,17;21 responded to OSAA, the frequency of this phenotype in normal Caucasians is only ~3%, and a much larger patient population would have to

be studied to determine conclusively whether this phenotype is associated with responsiveness or unresponsiveness to OSAA. It would also be of interest to study household contacts of osteosarcoma patients, because in this group the incidence of antibodies to OSAA is much higher (over 25%) than in noncontacts.

Finally, the interactive effect of Ig allotype-linked and MHC-linked genes, as has been demonstrated for susceptibility to experimental myasthenia gravis in mice (22) and for autoimmune chronic active hepatitis (23) and immune responses to bacterial antigens in man (12), may occur in osteosarcoma as well. In future studies, we plan to analyze both HLA antigens and Gm allotypes in a larger population of osteosarcoma patients, to determine whether there is any interactive effect of these two unlinked genetic systems on the pathogenesis of osteosarcoma and on immune responses to OSAA. This report is the first description of a strong association between immune responsiveness to a human cancer and Ig allotypes.

### Summary

Serum samples from 50 Caucasian patients with osteosarcoma were tested for the presence of antibodies to osteosarcoma-associated antigens (OSAA) and typed for nine Gm markers. A highly significant association was found between Gm 3;5,13,14 and unresponsiveness to OSAA, and between Gm 1,3,17;5,13,14,21 and responsiveness to OSAA. These results suggest the existence of complementary immune response genes which in the heterozygous condition permit a response to OSAA.

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